US
A19

GH
10/18/19

AMERICAN FERN JOURNAL

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

VOLUME 108 NUMBER 3 JULY-AUGUST 2018

THE AMERICAN FERN SOCIETY

Council for 2018

ERIC SCHUETTPELZ, National Museum of Natural History, Smithsonian Institution,
P.O. Box 37012, Washington DC 20013-7012 *President*

GEORGE YATSKIEVYCH, Plant Resources Center, University of Texas at Austin, Main Bldg,
Rm 127, 110 Inner Campus Dr, Stop F0404, Austin, TX 78712-1711 *President Elect*

MARY STENSVOLD, P. O. Box 1042, Sitka, Alaska 99835 *Secretary*

JAMES D. CAPONETTI, Div. of Biology, University of Tennessee, Knoxville, TN 37996-0830 *Treasurer*

JORDAN METZGAR, Massey Herbarium, 2119 Derring Hall, Virginia Tech,
Blacksburg, VA 24061 *Membership Secretary*

GEORGE YATSKIEVYCH, Plant Resources Center, University of Texas at Austin, Main Bldg,
Rm 127, 110 Inner Campus Dr, Stop F0404, Austin, TX 78712-1711 *Curator of Publications*

CHRISTOPHER H. HAUFLER, Department of Ecology and Evolutionary Biology,
University of Kansas, Lawrence, KS 66045 *Journal Editor*

DARLA HARRIS, 16240 Hartman Rd., Magnolia, TX 77355
JOAN N. E. HUDSON, Dept. of Biological Science, Sam Houston State University,
Huntsville, TX 77341-2116 *Bulletin Editors*

AMERICAN FERN JOURNAL

EDITOR

CHRISTOPHER H. HAUFLER ... Department of Ecology and Evolutionary Biology, University of Kansas,
Lawrence, KS 66045, e-mail: vulgare@ku.edu

MANAGING EDITOR

TOM A. RANKER........... Department of Botany, University of Hawai'i at Mānoa, Honolulu, HI 96822

ASSOCIATE EDITORS

JOSHUA P. DER.... Department of Biological Science, California State University, Fullerton, CA 62834
JOSE MARÍA GABRIEL Y GALÁN .... Dept. de Biología Vegetal I, Universidad Complutense de Madrid,
Madrid, Spain 28040
GARY K. GREER .... Biology Department, Grand Valley State University, Allendale, MI 49401
KLAUS MEHLTRETER .... Instituto de Ecología, A.C., Xalapa 91070, Ver., México
ROBBIN C. MORAN .... New York Botanical Garden, Bronx, NY 10458-5126
NATHALIE NAGALINGUM .... Institute for Biodiversity Science, California Academy of Science,
San Francisco, CA 94118
CARL ROTHFELS ..... Department of Integrative Biology, University of California, Berkeley, CA 64720
EMILY B. SESSA .... Department of Biology, University of Florida, Gainesville, FL 32611
MICHAEL SUNDUE .... Pringle Herbarium, The University of Vermont, Burlington, VT 05405
JAMES E. WATKINS, JR. .... Department of Biology, Colgate University, Hamilton, NY 13346

US
A19

GRAY HERBARIUM
LIBRARY

OCT 18 2019

American Fern Journal 108(3):65–75 (2018)
Published on 16 October 2018

Asplenium serratifolium (Aspleniaceae), a New Fern Species from Central Vietnam Based on Morphological and Molecular Evidence

KE-WANG XU
State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources,
School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong 510275, China;
Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166-0299, U.S.A.
e-mail: xukw6@mail2.sysu.edu.cn

LIANG ZHANG
Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany,
Chinese Academy of Sciences, Kunming, Yunnan 650201, China. e-mail: 549133356@qq.com

NGAN THI LU
CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization and
Ecological Restoration and Biodiversity Conservation Key Laboratory of Sichuan Province,
Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, Chengdu, Sichuan
610041, China; University of Chinese Academy of Sciences, Beijing 100049, China;
Department of Biology, Vietnam National Museum of Nature, Vietnam Academy of Science and
Technology, 18th Hoang Quoc Viet Road, Ha Noi, Vietnam. e-mail: vnmngan@gmail.com

LI-BING ZHANG*
Missouri Botanical Garden, 4344 Shaw Blvd., St. Louis, Missouri 631110, U.S.A.;
Chengdu Institute of Biology, Chinese Academy of Sciences: e-mail: Libing.Zhang@mobot.org

ABSTRACT.—*Asplenium serratifolium* (Aspleniaceae), a new fern species from central Vietnam, is described and illustrated. The new species is characterized by plants 10–18 cm tall, laminae pinnatipartite, lobe margins entire or with shallow teeth, and veins simple or forked. Molecular phylogenetic analysis based on five plastid markers (*atpB, rbcL, rps4, rps4-trnS,* and *trnL-F*) indicate that the new species is closely related to *A. ensiforme.*

KEY WORDS.—*Asplenium ensiforme*, pinnatipartite, fern phylogeny, Quang Tri Province

The largest fern genus, *Asplenium* L. (Aspleniaceae), is sub-cosmopolitan and comprises more than 700 species (Lin and Viane, 2013), of which 44 species are known to occur in Vietnam (Pham-Hoang, 1999). During fieldwork to survey ferns and lycophytes in central Vietnam in November 2014, two collections of a species of *Asplenium* were obtained from Quang Tri Province. The pinnatipartite lamina of this species distinctly differs from that of any other *Asplenium* known in Asia (Pham-Hoang, 1999; Lin and Viane, 2013; Lindsay and Middleton, 2012). Based on the investigation of morphological characters, literature, specimen comparisons, and phylogenetic analysis, we found that this species of *Asplenium* is new to science (Pham-Hoang, 1999; Lin and Viane, 2013; Lindsay and Middleton, 2012). We here describe this new species as *Asplenium serratifolium*.

---

* Corresponding Author

Material and Methods

Materials and taxon sampling.—We sequenced one individual from each of the two known populations of the new species, both from the Bac Huong Hoa Nature Reserve, Quang Tri, Vietnam. A total of 26 additional species of *Asplenium* were included in the molecular analyses based on morphological similarities and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) search results of the *rbcL* sequence of the new species with default settings in BLAST. Three important references, Schneider (2004), Schneider *et al.* (2017), and Ohlsen *et al.* (2015), were also consulted in choosing the ingroup taxa. Species of *Hymenasplenium* Hayata were used as outgroups because there are only two genera in the family generally recognized (Ohlsen *et al.*, 2015).

Morphological study.—The morphological description of the new species was based on the holotype and a paratype deposited in the Missouri Botanical Garden herbarium (MO). Measurement of the characteristics of roots, stipes, rachises, scales, and indusia was conducted using a micrometer under a dissecting microscope. Herbarium investigations were carried out at CDBI, MO, PHH, SYS, and VNMN and by using online images in the Chinese Virtual Herbarium (http://www.cvh.org.cn) provided by various Chinese herbaria and on those in JSTOR Global Plants (https://plants.jstor.org/) provided by herbaria worldwide.

DNA extraction, PCR amplification and sequencing.—Total genomic DNA was extracted from silica-gel-dried leaves using the modified 2×CTAB procedure of Doyle and Doyle (1987). DNA sequence data were obtained from five plastid markers: *atpB*, *rbcL*, *rps4*, *rps4-trnS* intergenic spacer, and *trnL-F* intergenic spacer. Primer sequences and PCR procedures followed Xu *et al.* (2018). Amplified fragments were purified with TIANquick Mini Purification Kits (TIANGEN). Purified PCR products were sequenced by TSINGKE Biological Technology (Guangzhou, China). DNA sequences of 23 *Asplenium* species were downloaded from GenBank. All sequences used in this study, together with their GenBank accession numbers, are listed in Appendix I.

Sequence alignment and phylogenetic analysis.—Sequences were edited and assembled using Sequencher V.4.14 (GeneCodes Corporation, Ann Arbor, Michigan, USA). Sequence alignment and adjustment were conducted manually with Bioedit (Hall, 1999). Phylogenetic relationships were analyzed through maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). MP tree searches were carried out for each data matrix in PAUP v. 4.0b10 (Swofford, 2002). Parsimony jackknife (JK) analyses (Farris *et al.*, 1996) were conducted using PAUP with the removal probability set to approximately 37%, and "jac" resampling emulated. Two hundred replicates were performed from different random addition sequence trees, with

TABLE 1. Best-fitting models, data matrices, and tree statistics for each of the analyses. Missing data include missing sequences, uncertain bases (N, R, Y, V, etc.), and gaps (-).

Locus	Selected model	# accessions	# missing (%)	# chars.
Plastid *atpB* gene	TIM1+G	6	84.5	1239
Plastid *rbcL* gene	TIM1+G	37	2.1	1252
Plastid *rps4* gene & *rps4-trnS* spacer	TPM1uf+I+G	18	63.5	1030
Plastid *trnL* intron & *trnL-F* spacer	TVM+G	18	62.4	384
Simultaneous	TIM1+I+G	37	50.4	3905

100 TBR searches per replicate and a maximum of 100 trees held per TBR search. ML tree searches and ML bootstrapping (BS) were performed using the web server RAxML-HPC2 on XSEDE v.8.2.10 on the CIPRES web server with 1,000 bootstrap replicates (Miller, Pfeiffer, and Schwartz, 2010). Bayesian inference was conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) on CIPRES (Miller, Pfeiffer, and Schwartz, 2010). Two runs of four Markov chain Monte Carlo chains were conducted, each beginning with a random tree and sampling one tree every 1000 generations of 10,000,000 generations. Convergence among chains was checked using Tracer ver. 1.4 (Rambaut and Drummond, 2007), and the first 25% was discarded as burn-in to ensure that stationarity had been reached. The remaining trees were used to calculate a 50% majority-rule consensus topology and posterior probabilities (PP).

jModelTest 2 (Guindon and Gascuel, 2003; Posada, 2008; Darriba *et al.*, 2012) was used to select the best-fitting likelihood model. The Akaike information criterion (Akaike, 1974) was used to select among models (Table 1), following Pol (2004) and Posada and Buckley (2004).

RESULTS AND DISCUSSION

The alignment of the combined plastid DNA datasets (*atpB*: 1,239 bp, *rbcL*: 1,252 bp, *rps4* and *rps4-trnS* intergenic spacer: 1,030 bp, *trnL-F* intergenic spacer: 384 bp) was 3,905 characters long (details concerning the datasets analyzed and statistics for the resulting trees are shown in Table 1) of which 535 were parsimony-informative and 388 were variable and parsimony-uninformative. In our plastid phylogeny, the three phylogenetic analyses (BI, ML, and MP) revealed similar topologies, with minor differences at some weakly supported branches, and all three trees indicated that the new species formed a strongly supported clade (MLBS: 100%, BIPP: 1, MPJK: 100%).

The two samples of the new species had identical DNA sequences, which were different from all other sampled species. In addition, the phylogenetic analyses indicate that the new species is closely related to *A. ensiforme* Wallich ex Hooker & Greville (Hooker and Greville, 1828; Fig. 1). Morphologically, *A. serratifolium* can be easily distinguished from *A. ensiforme* in having plants 10–18 cm tall, laminae pinnatipartite, and lobe margins entire or with shallow teeth, whereas *A. ensiforme* has plants 30–45 (–65) cm tall, laminae

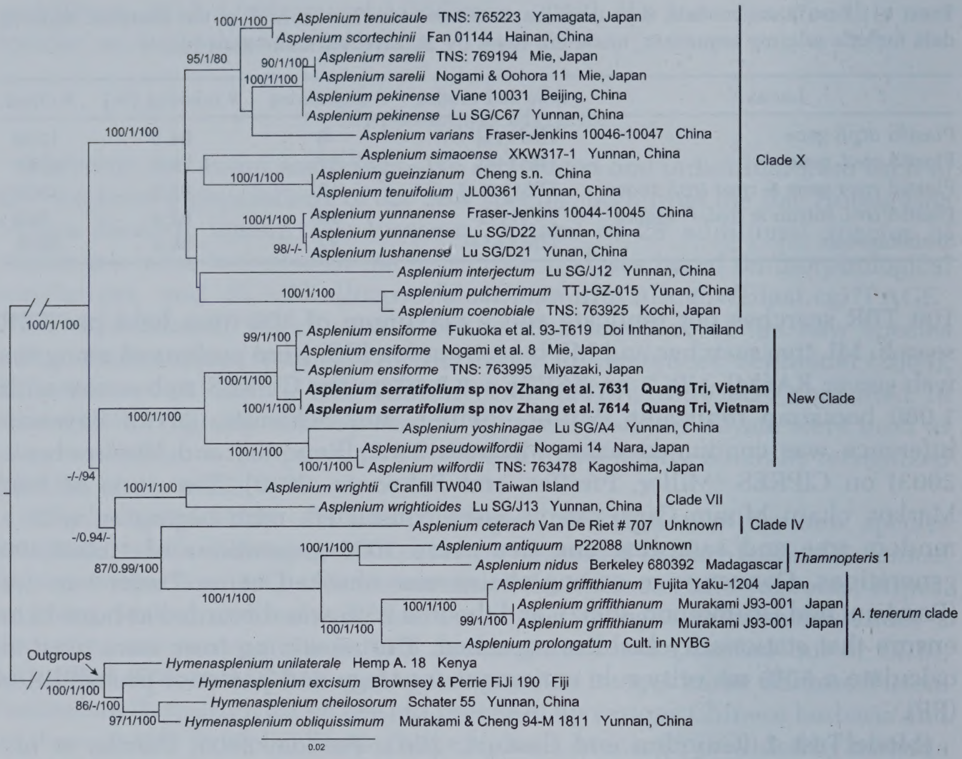
100/1/100 Asplenium tenuicaule TNS: 765223 Yamagata, Japan
Asplenium scortechinii Fan 01144 Hainan, China
95/1/80
90/1/100 Asplenium sarelii TNS: 769194 Mie, Japan
100/1/100 Asplenium sarelii Nogami & Oohora 11 Mie, Japan
Asplenium pekinense Viane 10031 Beijing, China
Asplenium pekinense Lu SG/C67 Yunnan, China
100/1/100
Asplenium varians Fraser-Jenkins 10046-10047 China
Asplenium simaoense XKW317-1 Yunnan, China
100/1/100 Asplenium gueinzianum Cheng s.n. China
Asplenium tenuifolium JL00361 Yunnan, China
100/1/100 Asplenium yunnanense Fraser-Jenkins 10044-10045 China
Asplenium yunnanense Lu SG/D22 Yunnan, China
98/-/- Asplenium lushanense Lu SG/D21 Yunan, China
Asplenium interjectum Lu SG/J12 Yunnan, China
100/1/100 Asplenium pulcherrimum TTJ-GZ-015 Yunan, China
Asplenium coenobiale TNS: 763925 Kochi, Japan
Clade X
100/1/100
99/1/100 Asplenium ensiforme Fukuoka et al. 93-T619 Doi Inthanon, Thailand
Asplenium ensiforme Nogami et al. 8 Mie, Japan
Asplenium ensiforme TNS: 763995 Miyazaki, Japan
100/1/100
Asplenium serratifolium sp nov Zhang et al. 7631 Quang Tri, Vietnam
100/1/100 Asplenium serratifolium sp nov Zhang et al. 7614 Quang Tri, Vietnam
100/1/100
New Clade
Asplenium yoshinagae Lu SG/A4 Yunnan, China
100/1/100 Asplenium pseudowilfordii Nogami 14 Nara, Japan
100/1/100 Asplenium wilfordii TNS: 763478 Kagoshima, Japan
-/-/94
100/1/100 Asplenium wrightii Cranfill TW040 Taiwan Island
Asplenium wrightioides Lu SG/J13 Yunnan, China
Clade VII
-/0.94/-
Asplenium ceterach Van De Riet # 707 Unknown
Clade IV
100/1/100 Asplenium antiquum P22088 Unknown
Asplenium nidus Berkeley 680392 Madagascar
Thamnopteris 1
87/0.99/100
Asplenium griffithianum Fujita Yaku-1204 Japan
100/1/100
100/1/100 Asplenium griffithianum Murakami J93-001 Japan
99/1/100 Asplenium griffithianum Murakami J93-001 Japan
100/1/100
Asplenium prolongatum Cult. in NYBG
A. tenerum clade
Outgroups
Hymenasplenium unilaterale Hemp A. 18 Kenya
100/1/100
Hymenasplenium excisum Brownsey & Perrie FiJi 190 Fiji
86/-/100
Hymenasplenium cheilosorum Schater 55 Yunnan, China
97/1/100 Hymenasplenium obliquissimum Murakami & Cheng 94-M 1811 Yunnan, China
0.02

FIG. 1. The phylogenetic position of *Asplenium serratifolium* based on five plastid markers (*atpB*, *rbcL*, *rps4*, *rps4-trnS*, *trnL-F*). The numbers associated with branches are maximum likelihood bootstrap support (MLBS), Bayesian posterior probability (BIPP), followed by maximum parsimony jackknife support (MPJK). "-" indicates MLBS < 85%, BIPP < 0.9%, or MPJK < 80%. The major clades (either new or following Ohlsen (2015)) are indicated on the right.

simple and entire to repand. Detailed comparison of the two species is listed in Table 2.

Only two populations of the new species in the Bac Huong Hoa Nature Reserve of Vietnam were found, while the related species *Asplenium ensiforme* is widely distributed in Asia (Lin and Viane, 2013).

TABLE 2. Morphological comparison between *Asplenium serratifolium* and *A. ensiforme*.

Character	*A. serratifolium*	*A. ensiforme*
Plant height	10–18 cm	30–45(–65) cm
Petiole length	1–6 cm	5–8(–15) cm
Rhizome scales	2–4 × 0.08–0.15 mm	Ca. 6 × 1 mm
Lamina	Pinnatipartite	Simple
Lobe margins	Entire or serrated	Entire to repand
Distribution	Central Vietnam	Southeast Asia
Veins	Simple or forked	Once-forked

TAXONOMIC TREATMENT

Asplenium serratifolium Li Bing Zhang & K.W. Xu, **sp. nov.** (Figs. 2, 3). TYPE: VIETNAM. **Quang Tri**: Huong Hoa District, Bac Huong Hoa Nature Reserve, Huong Viet Commune, Ta Rung Village, 1250 m, 16°48′N, 106°35′E, 22 Nov 2014, *Li-Bing Zhang, Liang Zhang & Ngan Thi Lu 7631* (holotype: MO!; isotypes: CDBI!, VNMN!).

Diagnosis.—Asplenium serratifolium somewhat resembles *A. ensiforme* by its stipes sulcate adaxially, midribs raised abaxially and sulcate adaxially, obscure veins, and sori in rows on two sides of midrib separately. The former has plants 10–18 cm tall, laminae pinnatipartite, lobe margins entire or with shallow teeth, and veins simple or forked, while the latter has plants 30–45(–65) cm tall, laminae simple, entire to repand, and veins once-forked.

Plants perennial, evergreen, 10–18 cm tall. Rhizomes erect, short, apices densely scaly; scales brown to dark brown, lanceolate, 2–6 × 0.18–0.5 mm, margins entire. Fronds simple and caespitose, herbaceous, yellowish green or grayish green when dry, subglabrous; stipes stramineous, sulcate adaxially, rounded to carinate abaxially, 1–6 cm, bases densely covered with scales, becoming subglabrous above, scales clathrate, brown, lanceolate, 2–4 × 0.08–0.15 mm, margins entire; laminae pinnatipartite, lanceolate, 5–12 × 0.6–1.8 cm, bases reduced and decurrent on stipes, apices acute to acuminate, widest at middle part of laminae, margins of lobes entire or serrate, teeth shallow and obtuse. Midribs stramineous, adaxially sulcate, raised abaxially, sparsely scaly, scales brown, lanceolate, ca. 0.08 × 0.8 mm, entire, glabrous; veins obscure, simple or forked. Sori linear, (0.1–) 0.2–0.5 cm, starting close to midribs at an angle of 25°–45° on acroscopic veinlets; indusia yellowish green or stramineous and becoming brown when dry, linear, papery, margins entire, opening toward midribs, persistent.

Geographical distribution.—Asplenium serratifolium is known from two locations within the Bac Huong Hoa Nature Reserve, Quang Tri, central Vietnam.

IUCN Red List category.—Only two populations and fewer than 50 individuals of *Asplenium serratifolium* are known. We did not find any other populations in the course of our three expeditions (from 2013–2015) to collect Vietnamese pteridophytes. As a result, a CR-Critically Endangered category is suggested based on current information available and following IUCN (the International Union for Conservation of Nature and Natural Resources) guidelines (IUCN, 2015).

Etymology.—Based on the Latin prefix, *serrati-*, serrate, and the Latin suffix, *-folium*, leaf, referring to the saw-toothed laminae of the new species.

Additional specimens examined (paratypes).—VIETNAM. **Quang Tri**: Huong Hoa District, Bac Huong Hoa Nature Reserve, Huong Viet Commune, Ta Rung Villige, Sa Mu Pass, elev. 1300 m, 16°48′N, 106°35′E, 21 Nov 2014, *Li-Bing Zhang, Liang Zhang & Ngan Thi Lu 7614* (CDBI!, MO!, VNMN!).

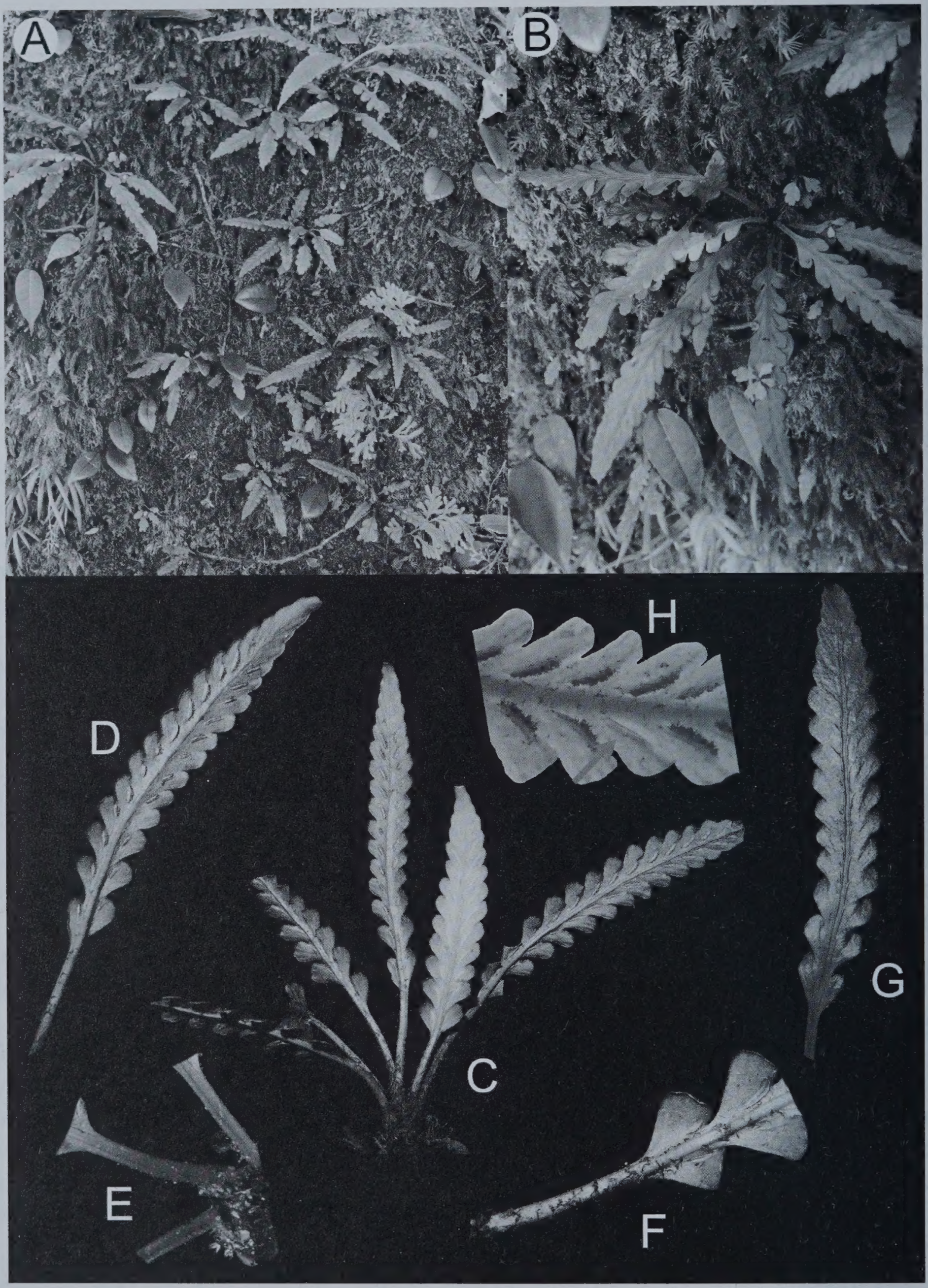

Fig. 2. *Asplenium serratifolium* sp. nov. A and B. Habit. C. Plant. D. Abaxial lamina. E. Sulcate stipe, adaxial view. F. Stipe scales. G. Adaxial lamina showing the sulcate midrib. H. Portion of abaxial lamina; red arrow shows the obscure, forked veins.

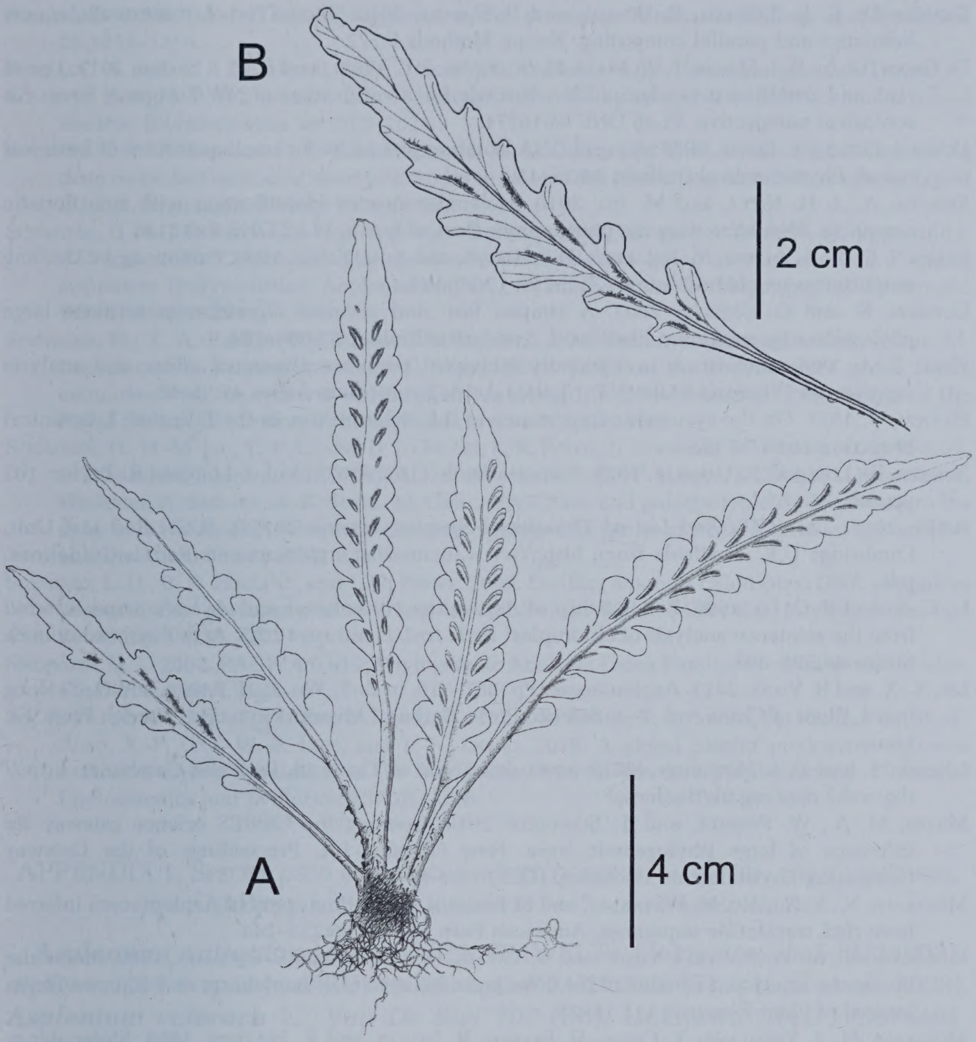

FIG. 3. *Asplenium serratifolium* sp. nov. A. Habit. B. Lamina, abaxial surface, showing simple and forked veins. (drawings by Lei Jiang, based on holotype at MO).

ACKNOWLEDGMENTS

This research was partially supported by grants from the National Natural Science Foundation of China (#31628002) and the National Geographic Society of the USA to L.-B.Z., funding from the China Scholarship Council (201706380093) and the Zhang Hong-Da (Chang Hung-Ta) Science Foundation at Sun Yat-sen University to K.-W.X. We thank Lei Jiang for the illustration and Chris Haufler, Carl Rothfels, and other two anonymous reviewers for helpful comments.

LITERATURE CITED

AKAIKE, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19:716–723.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9:772.

De Groot, G. A., H. J. During, J. W. Maas, H. Schneider, J. C. Vogel, and R. H. J. Erkens. 2011. Use of *rbcL* and *trnL-F* as a two-locus DNA barcode for identification of NW-European ferns: An ecological perspective. PLoS ONE 6:e16371.

Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

Ebihara, A., J. H. Nitta, and M. Ito. 2010. Molecular species identification with rich floristic sampling: DNA barcoding the pteridophyte flora of Japan. PLoS ONE 5:e15136.

Farris, J. S., V. A. Albert, M. Kallersjo, D. Lipscomb, and A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12:99–124.

Guindon, S. and O. Gascuel. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52:696–704.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Hayata, B. 1927. On the systematic importance of the stelar system in the Filicales, I. Botanical Magazine 41:697–718.

Hooker, W. J. and R. K. Greville. 1828. *Icones Filicum* (1827–1828) Vol. I. London: R. Taylor. 105 pp, tab. 71.

IUCN. 2015. *The IUCN Red List of Threatened Species*, version 2015-3. IUCN Red List Unit, Cambridge U.K. Available from: http://www.iucnredlist.org/documents/RedListGuidelines.pdf.

Li, C.-X. and S.-G. Lu. 2006. Relationship of *Asplenium yunnanense* and *A. lushanense* inferred from the sequence analysis of chloroplast *rbcL*, *trnL-F* and *rps4-trnS*. Acta Phytotaxonomica Sinica 44:296–303.

Lin, Y.-X. and R. Viane. 2013. Aspleniaceae. Pp. 267–316, in Z.-Y. Wu, P. H. Raven, and D.-Y. Hong (eds.), Flora of China, vol. 2–3. Science Press, Beijing / Missouri Botanical Garden Press, St. Louis.

Lindsay, S. and D. J. Middleton. (2012 onwards). Ferns of Thailand, Laos and Cambodia. http://rbg-web2.rbge.org.uk/thaiferns/

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. New Orleans, LA. Proceedings of the Gateway Computing Environments Workshop (GCE) 14:1–8.

Murakami, N., S. Nogami, M. Watanabe, and K. Iwatsuki. 1999. Phylogeny of Aspleniaceae inferred from *rbcL* nucleotide sequences. American Fern Journal 89:232–243.

Murakami, N., M. Watanabe, J. Yokoyama, Y. Yatabe, H. Iwasaki, and S. Serizawa. 1999. Molecular taxonomic study and revision of the three Japanese species of *Asplenium* sect. *Thamnopteris*. Journal of Plant Research 112:15–25.

Murakami, N., J. Yokoyama, X. Cheng, H. Iwasaki, R. Imaichi, and K. Iwatsuki. 1998. Molecular α-taxonomy of *Hymenasplenium obliquissimum* complex (Aspleniaceae) based on *rbcL* sequence comparisons. Plant Species Biology 13:51–56.

Ohlsen, D. J., L. R. Perrie, L. D. Shepherd, P. J. Brownsey, and M. J. Bayly. 2015. Phylogeny of the fern family Aspleniaceae in Australasia and the south-western Pacific. Australian Systematic Botany 27:355–371.

Pham-hoang, H. 1999. *An Illustrated Flora of Vietnam*, vol. 1. Ho Chi Minh City: Nha Xuat Ban Tre. pp. 143–154.

Pinter, I., F. Bakker, J. Barrett, C. Cox, M. Gibby, S. Henderson, M. Morgan-Richards, F. Rumsey, S. Russell, S. Trewick, H. Schneider, and J. Vogel. 2002. Phylogenetic and biosystematic relationships in four highly disjunct polyploid complexes in the subgenera *Ceterach* and *Phyllitis* in *Asplenium* (Aspleniaceae). Organisms, Diversity and Evolution 2:299–311.

Pol, D. 2004. Empirical problems of the hierarchical likelihood ratio test for model selection. Systematic Biology 53:949–962.

Posada, D. and T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53:793–808.

Posada, D. 2008. JModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

Rambaut, A. and A. J. Drummond. 2007. Tracer 1.4. Available: http://beast.bio.ed.ac.uk/Tracer.

Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Rothfels, C. J., A. Larsson, L. Y. Kuo, P. Korall, W. L. Chiou, and K. M. Pryer. 2012. Overcoming deep roots, fast rates, and short internodes to resolve the ancient rapid radiation of eupolypod II ferns. Systematic Biology 61:490–509.

Schneider, H., S. J. Russell, C. J. Cox, F. Bakker, S. Henderson, F. Rumsey, J. Barrett, M. Gibby, and J. C. Vogel. 2004. Chloroplast phylogeny of asplenioid ferns based on *rbcL* and *trnL-F* spacer sequences (Polypodiidae, Aspleniaceae) and its implications for biogeography. Systematic Botany 29:260–274.

Schneider, H., T. A. Ranker, S. J. Russell, R. Cranfill, J. M. Geiger, R. Aguraiuja, K. R. Wood, M. Grundmann, K. Kloberdanz, and J. C. Vogel. 2005. Origin of the endemic fern genus *Diella* coincides with the renewal of Hawaiian terrestrial life in the Miocene. Proceedings of the Royal Society of London B: Biological Sciences 272:455–460.

Schneider, H., H.-M. Liu, Y.-F. Chang, D. J. Ohlsen, L. R. Perrie, L. Shepherd, M. Kessler, D. N. Karger, S. Hennequin, J. Marquardt, S. Russell, S. Ansell, N. T. Lu, P. Kamau, J. LÓriga, L. Regalado, J. Heinrichs, A. Ebihara, A. R. Smith, M. Gibby. 2017. Neo- and paleopolyploidy contribute to the species diversity of *Asplenium*—the most species-rich genus of ferns. Journal of Systematics and Evolution 55:353–364.

Shepherd, L. D., B. R. Holland, and L. R. Perrie. 2008. Conflict amongst chloroplast DNA sequences obscures the phylogeny of a group of *Asplenium* ferns. Molecular Phylogenetics and Evolution 48:176–187.

Swofford, D. L. 2002. PAUP/ 4.0 b10: Phylogenetic Analysis Using Parsimony. Sinauer Associates, Sunderland, MA, USA.

Xu, K.-W., X.-M. Zhou, Q.-Y. Yin, L. Zhang, N. T. Lu, R. Knapp, T. T. Luong, H. He, Q. Fan, W.-Y. Zhao, X.-F. Gao, W.-B. Liao, and L.-B. Zhang. 2018. A global plastid phylogeny uncovers extensive cryptic speciation in the fern genus *Hymenasplenium* (Aspleniaceae). Molecular Phylogenetics and Evolution 127:203–216.

APPENDIX I. Species used in molecular phylogenetic analyses, their vouchers, and GenBank accession numbers. "—" means missing data.

Asplenium antiquum Makino, *P22088* (WELT), Unknown: *rbcL* EU240033, *atpB* —, *rps4 & rps4-trnS* EU240020, *trnL-F* EU240028 (Shepherd *et al.*, 2008). ***Asplenium ceterach*** L., *Van De Riet 707* (EB), Unknown: *rbcL* HQ676494, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* HQ676516 (de Groot *et al.*, 2011). ***Asplenium coenobiale*** Hance, *TNS: 763925* (TNS), Kochi, Japan: *rbcL* AB574855, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Ebihara *et al.*, 2010). ***Asplenium ensiforme*** Wall. ex Hook. & Grev., *TNS: 763995* (TNS), Miyazaki, Japan: *rbcL* AB574856, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Ebihara *et al.*, 2010). ***Asplenium ensiforme*** Wall. ex Hook. & Grev., *Nogami et al. 8* (KYO), Mie, Japan: *rbcL* AB014689, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Murakami *et al.*, 1999). ***Asplenium ensiforme*** Wall. ex Hook. & Grev., *Fukuoka et al. 93-T619* (KYO), Doi Inthanon, Thailand: *rbcL* AB014709, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Murakami *et al.*, 1999). ***Asplenium griffithianum*** Hook., *Fujita et al., Yaku-1204* (FU), Kagoshima, Japan: *rbcL* AB574857, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Ebihara *et al.*, 2010). ***Asplenium griffithianum*** Hook., *Murakami J93-001* (TI), Kagoshima, Japan: *rbcL* AB013252, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Murakami *et al.*, 1999a). ***Asplenium***

griffithianum Hook., *Murakami J93-001* (KYO), Kagoshima, Japan: *rbcL* AB014688, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Murakami *et al.*, 1999a). ***Asplenium interjectum*** Christ, *Lu SG/J 12* (PYU), Yunnan, China: *rbcL* AY545480, *atpB* —, *rps4 & rps4-trnS* AY725043, *trnL-F* AY725038 (Li and Lu, 2006). ***Asplenium laciniatum*** D. Don, *Cheng s.n.* (BM), China: *rbcL* AY549747, *atpB* —, *rps4 & rps4-trnS* AY549801, *trnL-F* AY549851 (Schneider *et al.*, 2005). ***Asplenium lushanense*** C. Chr., *Lu SG/D 21* (PYU), Yunnan, China: *rbcL* AY545481, *atpB* —, *rps4 & rps4-trnS* AY725042, *trnL-F* AY725033 (Li and Lu, 2006). ***Asplenium nidus*** L., *U.C. Botanical Garden 68.0392* (UC), Madagascar: *rbcL* AF525270, *atpB* —, *rps4 & rps4-trnS* AY549807, *trnL-F* AF525246 (Pinter *et al.*, 2002). ***Asplenium pekinense*** Hance, *Viane 10031*, Unknown: *rbcL* GU929864, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Unpublished data). ***Asplenium pekinense*** Hance, *Lu SG/C 67* (PYU), Yunnan, China: *rbcL* AY545479, *atpB* —, *rps4 & rps4-trnS* AY725040, *trnL-F* AY725037 (Li and Lu, 2006). ***Asplenium prolongatum*** Hook., *Cult. in NYBG* (UC), New York, US: *rbcL* AY549752, *atpB* —, *rps4 & rps4-trnS* AY549813, *trnL-F* AY549856 (Schneider *et al.*, 2005). ***Asplenium pseudowilfordii*** Tagawa, *Nogami 14* (KYO), Nara, Japan: *rbcL* AB014696, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Murakami *et al.*, 1999). ***Asplenium pulcherrimum*** (Baker) Ching ex Tardieu, *Ke-Wang Xu TTJ-GZ-015* (SYS), Guizhou, China: *rbcL* MH109142, *atpB* MH109136, *rps4 & rps4-trnS* MH109148, *trnL-F* (Unpublished data). ***Asplenium sarelii*** Hook., *TNS:769194* (TNS), Mie, Japan: *rbcL* AB574873, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Ebihara *et al.*, 2010). ***Asplenium sarelii*** Hook., *Nogami & Oohora 11*, Mie, Japan: *rbcL* AB014693, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Murakami *et al.*, 1999). ***Asplenium scortechinii*** Bedd., *Fan 01144*, Hainan, China: *rbcL* MH509434, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (this study). ***Asplenium serratifolium*** Li Bing Zhang & Ke-Wang Xu, *Zhang et al. 7631* (CDBI, MO, VNMN), Quang Tri, Vietnam: *rbcL* MH509432, *atpB* MH509430, *rps4 & rps4-trnS* MH509435, *trnL-F* MH509437 (this study). ***Asplenium serratifolium*** Li Bing Zhang & Ke-Wang Xu, *Zhang et al. 7614* (CDBI, MO, VNMN), Quang Tri, Vietnam: *rbcL* MH509433, *atpB* MH509431, *rps4 & rps4-trnS* MH509436, *trnL-F* MH509438 (this study). ***Asplenium simaoense*** Ke-Wang Xu, Li Bing Zhang & W. B. Liao, *Ke-Wang Xu XKW317-1* (SYS), Yunnan, China: *rbcL* MH109137, *atpB* MH109131, *rps4 & rps4-trnS* MH109143, *trnL-F* MH109149 (Unpublished data). ***Asplenium tenuicaule*** Hayata, *TNS: 765223* (TNS), Yamagata, Japan: *rbcL* AB574878, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Ebihara *et al.*, 2010). ***Asplenium tenuifolium*** D. Don, *Lei Jiang JL00361* (SYS), Yunnan, China: *rbcL* MH109141, *atpB* MH109135, *rps4 & rps4-trnS* MH109147, *trnL-F* MH109153 (Unpublished data). ***Asplenium varians*** Wall. ex Hook. & Grev., *Fraser-Jenkins 10046-10047* (BM), China: *rbcL* AY300147, *atpB* —, *rps4 & rps4-trnS* AY549802, *trnL-F* AY300094 (Schneider *et al.*, 2004). ***Asplenium wilfordii*** Mett. ex Kuhn, *TNS: 763478* (TNS), Kagoshima, Japan: *rbcL* AB574883, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Ebihara *et al.*, 2010). ***Asplenium wrightii*** D. C. Eaton ex Hook., *Cranfill TW040* (UC), Taiwan: *rbcL* AY549730, *atpB* —, *rps4 & rps4-trnS* AY549766, *trnL-F* AY549833 (Schneider *et al.*, 2005). ***Asplenium wrightioides*** Christ, *Lu*

SG/J13 (PYU), Yunnan, China: *rbcL* AY725031, *atpB* —, *rps4 & rps4-trnS* AY725044, *trnL-F* AY725032 (Li and Lu, 2006). ***Asplenium yoshinagae*** Makino, *Lu SG/A4* (PYU), Yunnan, China: *rbcL* AY725030, *atpB* —, *rps4 & rps4-trnS* AY725045, *trnL-F* AY725036 (Li and Lu, 2006). ***Asplenium yunnanense*** Franch., *Lu SG/D 22* (PYU), Yunnan, China: *rbcL* AY545482, *atpB* —, *rps4 & rps4-trnS* AY725041, *trnL-F* AY725034 (Li and Lu, 2006). ***Asplenium yunnanense*** Franch., *Fraser-Jenkins 10044-10045* (BM), China: *rbcL* AY300149, *atpB* —, *rps4 & rps4-trnS* AY549803, *trnL-F* AY300096 (Schneider *et al.*, 2004). ***Hymenasplenium cheilosorum*** (Kunze ex Mett.) Tagawa, *Schater 55* (GOET), Yunnan, China: *rbcL* JF832071, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Rothfels *et al.*, 2012). ***Hymenasplenium excisum*** (C. Presl) S. Lindsay, *Brownsey & Perrie FIJI 190* (WELT), Fiji: *rbcL* KP774884, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Ohlsen *et al.*, 2015). ***Hymenasplenium obliquissimum*** (Hayata) Sugimoto, *Murakami & Cheng 94-M 1811*, Yunnan, China: *rbcL* AB016186, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Murakami *et al.*, 1998). ***Hymenasplenium unilaterale*** (Lam.) Hayata, *Hemp 18* (BM), Kenya: *rbcL* AF240652, *atpB* —, *rps4 & rps4-trnS* —, *trnL-F* — (Pinter *et al.*, 2002).

American Fern Journal 108(3):76–94 (2018)
Published on 16 October 2018

Richness Patterns of Ferns Along an Elevational Gradient in the Sierra de Juárez, Oaxaca, Mexico: a Comparison with Central and South America

ADRIANA HERNÁNDEZ-ROJAS*
Faculty of Geography, Philipps-Universität Marburg, Biegenstraße 10, 35032 Marburg, Germany.
adriana.hernandezrojas@geo.uni-marburg.de, adric.rojas@gmail.com

MICHAEL KESSLER
Institut für Systematische und Evolutionäre Botanik, Universität Zürich, Zollikerstrasse 107, 8008 Zürich, Switzerland. michael.kessler@systbot.uzh.ch

THORSTEN KRÖMER
Centro de Investigaciones Tropicales (CITRO), Universidad Veracruzana, José María Morelos 44 y 46, Zona Centro, Xalapa, Veracruz, C.P. 91000, México. tkromer@uv.mx

CÉSAR CARVAJAL-HERNÁNDEZ
Instituto de Investigaciones Biológicas, Universidad Veracruzana, Av. Luis Castelazo Ayala s/n, Industrial Ánimas, Xalapa, Veracruz, México. ccarvajal@uv.mx

ANNA WEIGAND
Institut für Systematische und Evolutionäre Botanik, Universität Zürich, Zollikerstrasse 107, 8008 Zürich, Switzerland. anna.weigand@uzh.ch

JÜRGEN KLUGE
Faculty of Geography, Philipps-Universität Marburg, Biegenstraße 10, 35032 Marburg, Germany.
juegen.kluge@geo.uni-marburg.de

ABSTRACT.—The transition zone from the tropics to the subtropics in Mexico is an outstanding and poorly explored area of biotic overlap, and as such very interesting to evaluate how species richness patterns change in the latitudinal context. In order to assess alpha diversity patterns of ferns in this zone, we analyzed changes in their assemblages along an elevational gradient from 300 m to 3,000 m in the Sierra de Juárez, Oaxaca, and compared the results with similar studies conducted in Central and South America. We report the most detailed plot-based elevational gradient study on ferns in the region and provide detailed data on species abundances and elevational ranges. In 121 plots of 20 × 20 m, we found 195 fern species, with the highest species numbers recorded at mid-elevations (1,100–2,200 m) and lower richness at both gradient extremes. This hump-shaped pattern was mainly driven by epiphytes, which contributed about 40% to the total species richness and had wider elevational ranges than terrestrial species, which contributed 38% to the total richness, and were more or less constant at low to mid elevations about 2,500 m, followed by a gradual decline. Overall, richness per plot was low compared to other elevational gradients within the tropics closer to the Equator. This decline of species richness towards the subtropics and temperate regions appears to be rather abrupt within Central America, but additional data are needed to close the remaining gap of knowledge between Costa Rica and Mexico.

KEY WORDS.—Pteridophytes, alpha diversity, distribution, elevational range, Priority Terrestrial Region.

---

* Corresponding author

Ferns and lycophytes (although different lineages, henceforth called ferns) globally comprise about 11,000 species, thus being the second most diverse group of vascular plants on Earth (Moran, 1998; PPG I, 2016). With more than 1,000 recorded species, they are well represented in Mexico, which has one of the best-documented fern floras in the world (Mickel and Smith, 2004). The country is considered an epicenter for species radiations in some fern groups (principally groups specialized in seasonally dry habitats), and the states of Oaxaca, Chiapas, and Veracruz harbor the highest species numbers in the country (Krömer *et al.,* 2015; Mickel and Beitel, 1988; Mickel and Smith, 2004). Because fern taxonomy is well studied and non-woody life forms contribute up to 58% of the vascular plant richness in tropical forests (Gentry and Dodson, 1987; Linares-Palomino *et al.,* 2009), ferns represent a valuable group for ecological and biogeographic studies.

In this regard, elevational gradients are an interesting natural experiment, because they include major environmental changes within short horizontal distances, while dispersal limitation is minimized. Ferns have been widely studied along tropical elevational gradients in recent years, and show consistent general richness pattern at local and regional scales, with highest richness commonly found at mid-elevations and decreasing diversity towards both high and low elevations (Bhattarai, Vetaas, and Grytnes, 2004; Karger *et al.,* 2011; Kessler, 2001; Kessler *et al.,* 2011; Kluge, Kessler, and Dunn, 2006; Parris, Beaman, and Beaman, 1992; Salazar *et al.,* 2013; Watkins *et al.,* 2006).

With respect to major life-forms, this hump-shaped distributional trend in tropical mountains is commonly driven by epiphytes, which are extraordinarily rich in multi-structured tree crowns within the humid cloud condensation layer between 1,000–3,000 m (Acebey, Krömer, and Kessler, 2017; Kessler, 2000; Kluge and Kessler, 2005; Krömer *et al.,* 2013; Watkins *et al.,* 2006).

However, how elevational fern richness changes with latitude is less well explored, and two alternative hypotheses have been proposed. The first assumes that richness peaks along elevational gradients gradually shift towards lower elevations with increasing distance from the Equator in parallel with decreasing temperatures. Thus, Kessler *et al.* (2011) showed that the maximum richness of ferns along elevational gradients in tropical wet environments is situated at around 15–17°C mean annual temperature, and this level should be found at lower elevations at higher latitudes. Alternatively, when comparing various fern transects from Bolivia to Mexico, Salazar *et al.* (2013) found that overall elevational richness is strikingly constant between Costa Rica and northern Bolivia. The major shift towards lower species richness was found at the transition from tropical to subtropical climates. However, this decline was not linked to a downward shift in the elevation of the richness peak, but rather reflected a general decline of species richness, as also found by Krömer *et al.* (2013), Acebey, Krömer, and Kessler (2017), and Carvajal-Hernández *et al.* (2017) in the regions of Los Tuxtlas and central Veracruz, in the southeast of Mexico. Apart from these publications, no other suitable transects have been studied thus far to allow a fine-scale assessment of

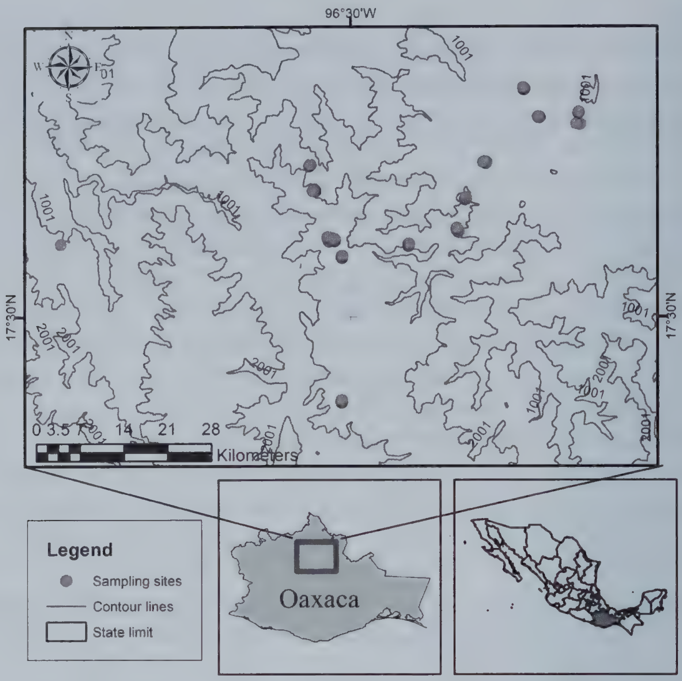

FIG. 1. Study area in the Sierra de Juárez, Oaxaca, Mexico, showing the locations of the sampling sites (dots).

the change of species richness patterns at the Mexican transition zone (MTZ, *sensu* Halffter and Morrone, 2017) between tropical and subtropical climates.

In the present study, we attempt to close a gap in our knowledge about elevational species richness patterns of ferns starting with the analysis of an extensive elevational gradient in the Sierra de Juárez, Oaxaca, inside the MTZ by presenting plot-based species richness data together with an annotated species list with detailed information on abundance and life form assignments. Specifically, we ask the following questions:

1. How does local species richness of ferns change with elevation?
2. Is the elevational pattern of species richness and ranges different between both major life forms (terrestrials and epiphytes)?
3. Is the total and maximum species richness significantly lower in magnitude and elevation compared to other studies performed in Central and South America?

METHODS

Study area.—The study area was located in southeastern Mexico in the Sierra de Juárez (also called Sierra Norte), Oaxaca, and the majority of the localities belong to the Chinantla region (Fig. 1, additional plot information in

Appendix 1). The Sierra de Juárez has been declared a Priority Terrestrial Region due to its species and ecosystem richness (Arriaga *et al.*, 2000), and thus is an important area for conservation. The eastern slope of the Sierra de Juárez hosts a very rich and one of the most distinctive floras in Mexico (Meave *et al.*, 2017) and represents the northernmost location within the Neotropics, where a large and well-preserved elevational gradient of humid tropical forest still remains (Boyle, 1996). There is an abrupt elevational increase in this area in less than 30 km from Valle Nacional (ca. 65 m) to the top of Humo Chico Mountain (ca. 3,200 m; Meave, Rincón, and Romero-Romero, 2006), called the—fern hunter‘s paradise by John Mickel (1965) during fieldwork to prepare the first treatment about ferns from Oaxaca (Mickel and Beitel 1988). Given the extensive deforestation elsewhere in Mexico (Rosete-Vergés *et al.*, 2014), the presence of this gradient of humid montane forest in Oaxaca is remarkable (Rzedowski and Palacios-Chávez, 1977). Despite a generally good conservation status above 1,000 m, human activities are leaving an important footprint in the lower parts of the area (Meave *et al.*, 2017). In addition, the Sierra is periodically impacted by strong winds from the Gulf coast (>80 km/h), which disturb the forest structure (Alfonso-Corrado *et al.*, 2017). There are few general studies on vegetation along the whole gradient in the Sierra de Juárez (Boyle, 1996; Meave, Rincón, and Romero-Romero, 2006; Rzedowski and Chávez, 1977; Rincón-Gutierrez, 2007). However, a recently published extensive floristic survey in the region reported 130 fern species along an elevational range from 250–3,020 m (Meave *et al.*, 2017). Forest zonation (with variation) begins in the lowlands with tropical rain forest from 300–1,000 m, followed by a transitional forest zone from 1,100–1,300 m, and cloud forest at 1,400–2,000 m (sometimes to 2,500 or more). Within the cloud forest belt, there are areas of forest dominated by *Oreomunnea mexicana*. Above 2,500 m there is *Quercus* forest and above 3,000 m elfin forest forms the treeline ecotone at around 3,100 m.

Climate.—La Chinantla is considered a hyperhumid region in Mexico (Meave, Rincón, and Romero-Romero, 2006, Meave *et al.*, 2017), but there are few climatic records for localities in the Sierra de Juárez. The climate at the lowest elevation is hot (>22°C) changing to a warm climate (18–22°C) higher up, and temperate at higher elevations (5–12 °C) (Meave, Rincón, and Romero-Romero, 2006). Mean annual precipitation is around 3,590 mm at the lower part of the gradient (Valle Nacional), 5,800 mm in the middle (La Esperanza), and 3,240 mm at the upper part at the locality of Humo Chico (Meave, Rincón, and Romero-Romero, 2006). Due to the scarcity of meteorological records in the study area, we extracted ecologically important temperature and precipitation data (Fig. 2) from the global climate dataset CHELSA (Karger *et al.*, 2017).

Fern sampling.—A total of 121 plots were inventoried at 15 sites along an elevational gradient between 300 m and 3,000 m at elevational intervals of 200–400 m (Appendix 1). At each elevation, eight plots of 20 × 20 m were sampled with a consistent, standardized methodology (Karger *et al.*, 2014; Kessler and Bach, 1999). The plots were established in natural zonal forest,

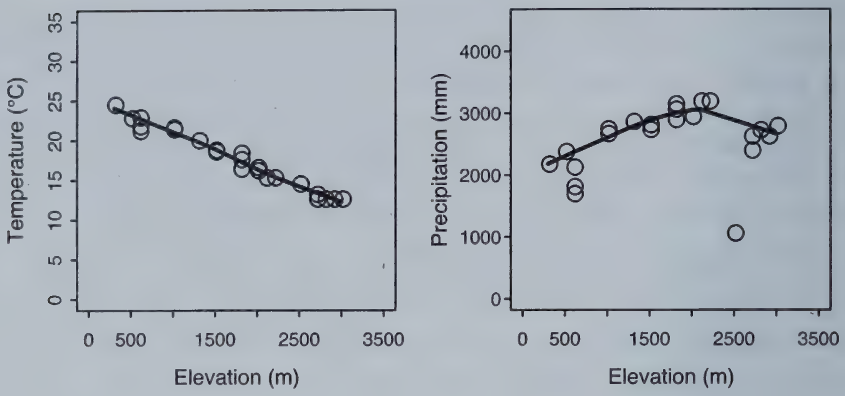

FIG. 2. Elevational trends of (a) mean annual temperature and (b) precipitation. Data derived from the CHELSA data set (Karger *et al.*, 2017) for each plot of the elevational transect in the Sierra de Juárez, Oaxaca, Mexico. Trend lines were set with locally weighted regressions (LOWESS).

avoiding special structural features like canopy gaps, ridges, ravines, riparian areas, treefall gaps, landslides, and other disturbed areas whenever possible. In each plot, all fern species and their abundances were recorded for terrestrials (growing on soil, rocks, and dead wood) and epiphytes. In most cases, the abundance (i.e., number of individuals of a species) is easy to assess, exceptions are only small species with distant fronds on long creeping and interwoven rhizomes (e.g., Hymenophyllaceae). Because in these cases exact numbers of individuals can only be obtained by destructive sampling, we treated a species patch as one individual in order not to overestimate the abundance of these species. Epiphytes were sampled up to heights of eight m, with trimming poles and recorded using binoculars at greater heights, by climbing lower parts of trees, and by searching recently fallen trees and branches within and adjacent to the plots (Gradstein *et al.*, 2003).

Samples of all fern species were collected and deposited in the University Herbarium, University of California (UC) in Berkeley, USA, herbarium XAL of the Instituto de Ecología, A. C. (Xalapa, Veracruz, Mexico) and herbarium MEXU of the Universidad Autónoma de México (Mexico City, Mexico). Collections were identified by the first author and Alan R. Smith (UC). Taxonomy primarily followed Mickel and Smith (2004) and the current classification for lycophytes and ferns established by the Pteridophyte Phylogeny Group (PPG I, 2016) and Hassler (2018).

Data analysis.—We assessed plot-based alpha-diversity for all species and separately for the two major lifeforms (terrestrials and epiphytes) and described their trends by locally weighted regressions (LOWESS). In order to assess whether the elevational ranges of terrestrials and epiphytes were different, we used a Wilcoxon signed-rank test. We considered a species as belonging to both life forms when more than 20% of the total number of individuals occurred in each life form. The frequencies of these three categories of life forms were compared with an analysis of variance (ANOVA)

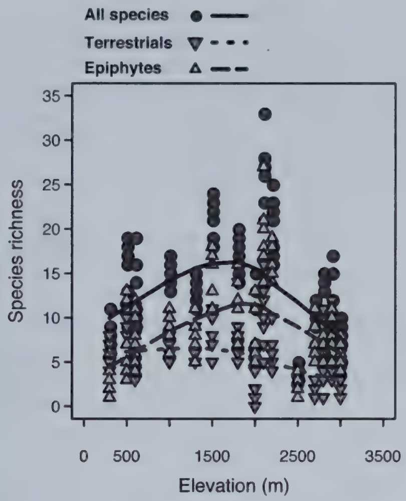

FIG. 3. Richness patterns of ferns (number of species per plot) along the elevational gradient in Sierra de Juárez, Oaxaca, Mexico. Values are given for all species (dots, continuous line), and separately for terrestrials (downwards-pointing triangles, short-dashed lines) and epiphytes (upwards-pointing triangles, long-dashed lines). Trend lines were set with locally weighted regressions (LOWESS).

and a Tukey post-hoc test ($P< 0.001$). We compared species richness per plot of our gradient with other similar transects from Bolivia, Ecuador, Costa Rica, and Mexico based on Kessler (2000, 2001), Kluge and Kessler (2005), Krömer *et al.* (2013), Salazar *et al.* (2013), and Carvajal-Hernández *et al.* (2017). To test the differences in mean plot richness of all transects, we used an analysis of variance and a Tukey post-hoc test ($P< 0.001$). Because the general objective of this work and the general richness pattern was not altered, we included atypical data from the site at 2,500 m located on the other side of the mountain chain (leeward), which was excluded only in the comparison between transects. All data analyses were conducted in the statistical package R (R Core Team, 2016.

RESULTS

In the 121 plots, we recorded 195 species of ferns and lycophytes belonging to 60 genera and 21 families, including two new records for Oaxaca State, *Ctenitis salvinii* and *Phlegmariurus capillaris,* as well as one species recently described as new to science (*Ceradenia sacksii*; Sundue, 2017) (TABLE 1). The most species-rich genera were *Elaphoglossum* (20 species), *Asplenium* (18), and *Pleopeltis* and *Hymenophyllum* (12 each). The most species-rich families were Polypodiaceae (49), Dryopteridaceae (35) and Hymenophyllaceae (25).

Fern species richness followed a hump-shaped elevational pattern with highest species numbers per plot at mid-elevations between 1,500 and 2,100 m and a marked decline towards both low and high elevations (Fig. 3). The

TABLE 1. Species list of lycophytes and ferns recorded along the elevational gradient in the Sierra de Juárez, Oaxaca, Mexico. Data are presented with the minimum (**Min**) and maximum (**Max**) observed elevational distribution, frequency of the species (**F**; the number of plots in which the species was recorded), the mean number of individuals per plot (**N**), and substrate including the life form: **t**: terrestrial, **he**: hemiepiphytic, **e**: for epiphytes comprising the Johansson (1974) zonation in which the epiphytes occurred (from zone 1 to zone 5), **dw**: dead wood, and **r**: rocks. Species marked with ^{n} are new records for Oaxaca and with * were found outside or close to the investigated plots.

Taxa	Min	Max.	N	F	Life form
Lycopodiaceae					
^{n}*Phlegmariurus capillaris* (Sodiro) B. Øllg.	2100	2100	1.00	1	e4
**Phlegmariurus cuernavacensis* (Underw. & F.E. Lloyd) B. Øllg.	2800	2800	1.00	1	e4
Phlegmariurus dichotomus (Jacq.) W.H.Wagner	500	500	1.00	2	e4
Phlegmariurus linifolius (L.) B.Øllg.	1000	2200	1.67	6	e1, e2, e3, e4
Phlegmariurus myrsinites (Lam.) B. Øllg.	2000	2100	1.33	3	e2, e3
Phlegmariurus pringlei (Underw. & F.E. Lloyd) B. Øllg.	1500	2200	4.00	4	e1, e2, e3, e4
Phlegmariurus taxifolius (Sw.) Á. Löve & D. Löve	600	2000	1.89	9	(r), e2, e3, e4, e5
Phlegmariurus wilsonii (Underw. & F.E.Lloyd) B.Øllg.	1500	1500	1.00	1	e3
Selaginellaceae					
Selaginella aff. *subrugosa* Mickel & Beitel	1800	1800	3.20	5	t
Selaginella flexuosa Spring	1000	2100	10.83	6	t, dw
Selaginella mosorongensis Hieron.	300	600	16.11	19	t, dw, r
Selaginella oaxacana Spring (GCI)	1300	1500	27.88	8	t
Selaginella subrugosa Mickel & Beitel	1300	1300	4.00	3	t
Selaginella sp.	600	600	7.50	2	r
Aspleniaceae					
Asplenium abscissum Willd.	500	600	13.67	3	(t), r, e1, e2, e3, e4
Asplenium auriculatum Sw.	1500	1500	11.00	2	dw, e1, e2, e3, e4
Asplenium cirrhatum Rich. ex Willd.	1300	2100	44.17	6	t, dw, r, e1, e2
Asplenium cristatum Lam.	500	500	1.00	1	rc
Asplenium cuspidatum Lam.	500	1800	41.63	8	(t), dw, r, e1, e2, e3, e4
Asplenium fragrans Sw.	1500	2700	14.00	6	dw, r, e1, e2, e3
Asplenium hallbergii Mickel & Beitel	2900	2900	94.00	2	r, e1
Asplenium harpeodes Kunze	2100	2100	7.00	1	e2
Asplenium miradorense Liebm.	1500	2100	9.75	4	t, dw, r, e1, e2
Asplenium monanthes L.	2500	2700	1.80	5	t, r, e1
Asplenium polyphyllum Bertol.	2700	2700	2.00	1	e2
Asplenium pteropus Kaulf.	1000	1000	2.00	1	e2
Asplenium salicifolium L.	500	500	1.50	2	e3
Asplenium radicans L.	1800	1800	1.00	2	t, e1
Asplenium serratum L.	300	600	52.53	15	(t), dw, r, e2, e3
Asplenium uniseriale Raddi	1300	1300	5.50	2	t
Asplenium sp.(cf. A. uniseriale Raddi)	2100	2100	1.00	1	t
Hymenasplenium riparium (Liebm.) L. Regalado & Prada	1000	1300	5.75	4	t, e2, e3, e4

TABLE 1. Continued.

Taxa	Min	Max.	N	F	Life form
Athyriaceae					
Diplazium diplazioides (Klotzsch & H.Karst.) Alston	1000	2100	7.50	8	t
Diplazium franconis Liebm.	2100	2100	3.00	2	t
Diplazium lonchophyllum Kunze	600	600	3.00	3	t
Diplazium plantaginifolium (L.) Urb.	600	600	14.00	2	t
Diplazium striatum (L.) C. Presl	1000	1000	5.00	1	t
Diplazium ternatum Liebm.	1000	2100	12.10	20	t, (e1)
Blechnaceae					
Austroblechnum wardiae (Mickel & Beitel) Gasper & V.A.O.Dittrich	2100	2100	1.00	1	t
Lomaridium ensiforme (Liebm.) Gasper & V.A.O.Dittrich	1500	2200	4.63	8	t, (e1), (e2)
Lomaridium fragile (Liebm.) Gasper & V.A.O. Dittrich	1500	2000	2.75	4	(t), e1, e2, e3
Parablechnum falciforme (Liebm.) Gasper & Salino	1800	2200	5.00	8	t, (e1)
Cyatheaceae					
Cyathea aristata Domin	1500	1500	2.50	2	t
Cyathea divergens Kunze	1500	1500	4.00	1	t
Cyathea firma (Bak.) Domin	1500	1500	3.50	2	t
Cyathea fulva (M. Martens & Galeotti) Fée	1500	2200	3.12	26	t, (e1)
Cyathea liebmannii Domin	1000	1000	20.00	1	t
Cyathea myosuroides (Liebm.) Domin	1000	1000	9.00	1	t, (e1), (e3)
Cyathea salvinii (Hook.) Domin	1500	2000	8.25	4	t
Cyathea schiedeana Domin	1000	1500	14.00	17	t
Sphaeropteris horrida (Liebm.) R.M. Tryon	1000	1000	2.00	1	t
Cyathea sp.	2100	2100	3.00	1	t
Dennstaedtiaceae					
Histiopteris incisa (Thunb.) J. Sm.	2000	2000	2.00	1	t
Hypolepis nigrescens Hook.	1000	1000	2.00	1	t
Pteridium feei (W. Schaffn. ex Fée) Faull	2500	2900	15.67	12	t
Dicksoniaceae					
Lophosoria quadripinnata (J.F.Gmel.) C.Chr.	2100	2200	3.75	4	t
Dryopteridaceae					
Arachniodes denticulata (Sw.) Ching	1300	2200	32.94	36	t, dw, r, (e1)
Bolbitis portoricensis (Spreng.) Hennipman	500	500	18.00	1	t
Ctenitis sp. (cf. *C. excelsa* (Desv.) Proctor)	300	1300	3.86	7	t, r
Ctenitis hemsleyana (Baker ex Hemsl.) Copel.	2000	2200	9.00	6	t, e1
Ctenitis melanosticta (Kunze) Copel.	500	500	7.60	5	t, r
Ctenitis nigrovenia (Christ) Copel.	300	300	6.00	4	t
[n]*Ctenitis salvinii* (Baker) Stolze	600	600	5.50	2	t, r
Dryopteris nubigena Maxon & C.V. Morton	2800	3000	5.25	4	r, (e1)
Dryopteris wallichiana (Spreng.) Hyl.	2700	3000	32.88	16	t, r
Elaphoglossum affine (M.Martens & Galeotti) T. Moore	2800	2900	27.80	5	t, r, e1

TABLE 1. Continued.

Taxa	Min	Max.	N	F	Life form
Elaphoglossum alan-smithii Mickel	2100	2200	6.25	4	e1, e2, e3, e4, e5
Elaphoglossum dissitifrons Mickel	2000	2900	11.71	17	t, e1, e2, e3, e4, e5
Elaphoglossum glabellum J.Sm.	1000	1000	1.00	1	e1
Elaphoglossum glaucum T. Moore	1800	3000	13.41	22	t, r, e1, e2, e3, e4
Elaphoglossum sp. (cf. *E. glaucum* T. Moore)	2100	2200	23.00	2	e1, e2, e3, e4
Elaphoglossum guatemalense (Klotzsch) T. Moore	1000	2000	7.43	21	t, dw, e1, e2, e3, e4, e5
Elaphoglossum leonardii Mickel	2800	2800	34.00	2	r
Elaphoglossum lonchophyllum (Fée) T. Moore	1500	2100	10.33	3	e1, e2, e3
Elaphoglossum muscosum (Sw.) T. Moore	1500	1500	1.00	1	e2
Elaphoglossum paleaceum (Hook. & Grev.) Sledge	2100	2200	6.64	11	e1, e2, e3, e4, e5
Elaphoglossum parduei Mickel	2000	2000	5.80	5	e1, e2, e3
Elaphoglossum peltatum (Sw.) Urb.	1500	2200	18.25	24	t, dw, rc, e1, e2, e3, e4, e5
Elaphoglossum petiolatum (Sw.) Urb.	2900	2900	1.00	2	e1, e2, e3, e4
Elaphoglossum pringlei (Davenp.) C.Chr.	2500	2500	8.33	3	t, dw, r
Elaphoglossum sartorii (Liebm.) Mickel	600	2200	22.17	35	t, dw, r, e1, e2, e3, e4
Elaphoglossum seminudum Mickel	2200	2200	80.25	4	t, e1
Elaphoglossum squamipes (Hook.) T. Moore	2700	2900	4.00	3	(t), dw, r, e1, e2
Elaphoglossum tenuifolium (Liebm.) T. Moore	2800	2900	7.80	5	t, r
Elaphoglossum vestitum (Schltdl. & Cham.) Schott	600	2100	17.57	37	e1, e2, e3, e4, e5
Elaphoglossum sp.	1000	1000	2.00	1	e2
Lastreopsis effusa (Sw.) Tindale	600	600	2.00	1	t, r
Megalastrum mexicanum R.C. Moran & J. Prado	600	600	13.25	4	t
Polystichum hartwegii (Klotzsch) Hieron	1800	2700	7.50	8	t
Polystichum mickelii A.R. Sm.	1300	1300	2.33	3	t, r
Stigmatopteris longicaudata C. Chr.	1500	1500	63.33	3	t
Gleicheniaceae					
Diplopterygium bancroftii (Hook.) A.R. Sm.	1800	1800	2.00	1	t
Hymenophyllaceae					
Abrodictyum rigidum (Sw.) Ebihara & Dubuisson	1000	1800	26.73	11	t, r
Didymoglossum hymenoides (Hedw.) Copel.	1300	1300	2.50	2	e1, e2
Didymoglossum kraussii (Hook. & Grev.) C. Presl	300	500	3.33	9	dw, e1, e3
Didymoglossum ovale E. Fourn.	500	500	3.00	1	r, e1
Didymoglossum reptans (Sw.) C. Presl	1500	2100	10.00	12	t, dw, r, e1, e2, e3
Hymenophyllum asplenioides Sw.	1500	2200	4.88	8	(t), e1, e2
* *Hymenophyllum brevistipes* Liebm.	1200	1200	1.00	1	e2
Hymenophyllum ectocarpon Fée	1800	3000	10.73	40	t, dw, r, e1, e2, e3
Hymenophyllum fragile (Hedw.) C.V. Morton	2100	2200	7.88	8	t, dw, r, e1, e2

TABLE 1. Continued.

Taxa	Min	Max.	N	F	Life form
Hymenophyllum fucoides Sw.	2100	2200	11.88	8	t, e1, e2
Hymenophyllum hirsutum (L.) Sw.	1000	1500	3.33	3	e1, e2, e3
Hymenophyllum lanatum Fée	1500	2200	2.15	13	(t), e1, e2, e3
Hymenophyllum myriocarpum Hook.	2000	2900	18.95	20	t, dw, e1, e2, e3, e4
Hymenophyllum polyanthos (Sw.) Sw.	1000	2000	3.75	16	t, dw, r, e1, e2, e3
Hymenophyllum tegularis (Desv.) Proctor & Lourteig	2800	2800	4.00	1	r
Hymenophyllum trapezoidale Liebm.	1500	2200	9.21	14	t, r, e1, e2, e3
Hymenophyllum tunbrigense Sm.	3000	3000	13.00	1	t, dw, e1
Hymenophyllum undulatum (Sw.) Sw.	1500	1800	5.40	5	e1, e2, e3
Polyphlebium capillaceum (L.) Ebihara & Dubuisson	1000	2100	2.80	5	dw, e1, e2
Polyphlebium hymenophylloides (Bosch) Ebihara & Dubuisson	1800	2200	2.86	7	t, dw, e1, e2
Polyphlebium pyxidiferum (L.) Ebihara & Dubuisson	500	1500	1.50	4	r, e1, e2, e4
Trichomanes lucens Sw.	1800	2000	1.00	2	t
Trichomanes polypodioides L.	1000	1500	43.00	3	t, e2
Trichomanes crispum L.	1300	1300	3.00	1	e1
Vandenboschia collariata (Bosch) Ebihara & K. Iwats.	600	600	3.67	3	r, e1
Vandenboschia radicans (Sw.) Copel.	1300	1500	6.20	5	t, dw, r, e1, e2
Lindsaeaceae					
Lindsaea arcuata Kunze	1000	1500	4.08	13	t
Lindsaea lancea Christ	1000	1000	1.00	1	t
Lomariopsidaceae					
Lomariopsis mexicana Holttum	1300	1300	2.00	1	t, he,(e1),(e2)
Lomariopsis recurvata Fée	300	600	4.64	11	t, he, (r),(e1), (e2), (e3)
Lygodiaceae					
Lygodium heterodoxum Kunze	300	300	1.50	2	t, he, (e1)
Marattiaceae					
Danaea moritziana C. Presl	1000	1300	7.50	4	t
Marattia excavata Underw.	1500	1500	1.00	3	t
Marattia weinmanniifolia Liebm.	2100	2100	2.00	4	t
Plagiogyriaceae					
Plagiogyria pectinata (Liebm.) Lellinger	2800	3000	34.50	12	t, (e2)
Polypodiaceae					
Alansmia cultrata (Willd.) Moguel & M. Kessler	1800	2900	19.64	14	r, e1, e2, e3
Campyloneurum xalapense Fée	600	1500	10.22	18	t, dw, e1, e2, e3, e4, e5
Campyloneurum amphostenon (Kunze ex Klotzsch) Fée	2100	2900	2.75	4	e2
Campyloneurum angustifolium (Sw.) Fée	300	1000	5.73	22	t, e1, e2, e3, e4, e5
Ceradenia oidiophora (Mickel & Beitel) A.R. Sm.	2100	2100	2.00	2	e1, e2
^{n}*Ceradenia sacksii* Sundue	2800	2800	20.00	2	r
Cochlidium linearifolium Maxon ex C. Chr.	1000	3000	37.11	37	e1, e2, e3, e4, e5
Galactodenia delicatula (M.Martens & Galeotti) Sundue & Labiak	2000	3000	5.77	22	e1, e2, e3, e4
Melpomene leptostoma (Fée) A.R. Sm. & R.C. Moran	1500	2200	7.25	24	e1, e2, e3, e4, e5

Table 1. Continued.

Taxa	Min	Max.	N	F	Life form
Melpomene moniliformis (Lag. ex Sw.) A.R. Sm. & R.C.Moran	2800	2900	63.73	11	t, dw, r, e1, e2, (e4)
Melpomene pilosissima (M.Martens & Galeotti) A.R.Sm. & R.C. Moran	2800	2800	5.00	1	e2
Melpomene xiphopteroides (Liebm.) A.R. Sm. & R.C.Moran	1000	2000	3.20	5	e1, e2, e3, e5
**Moranopteris basiattenuata* (Jenman) R.Y. Hirai & J.Prado	2400	2400	1.00	1	e1
Moranopteris trichomanoides (Sw.) R.Y. Hirai & J.Prado	1800	1800	2.00	1	e2
**Mycopteris semihirsuta* (Klotzsch) Sundue	2400	2400	1.00	1	e1
Niphidium crassifolium (L.) Lellinger	300	1300	3.47	17	t, e1, e2, e3, e4
Pecluma longepinnulata (E. Fourn.) F.C. Assis & Salino	1300	2100	10.53	15	t, dw, r, e1, e2, e3, e4
Pecluma atra (A.M.Evans) M.G. Price	300	300	4.33	3	r, e2, e3
Pecluma consimilis (Mett.) M.G. Price	500	600	5.20	5	t, dw, r, e1, e2, e3, e4
Pecluma dispersa (A.M.Evans) M.G. Price	500	500	2.00	1	e1
Pecluma divaricata (E.Fourn.) Mickel & Beitel	500	500	7.00	2	e2, e3
Pecluma hartwegiana (Hook.) F.C. Assis & Salino	2700	3000	78.24	25	dw, r, e1, e2, e3, e4, e5
Pecluma rhachipterygia (Liebm.) F.C. Assis & Salino	600	1300	8.00	8	t, rc, e1, e2
Pecluma sursumcurrens (Copel.) M.G. Price	500	500	1.00	1	dw, e1, e2, e3, e4, e5
Phlebodium areolatum (Humb. & Bonpl. ex Willd.) J. Sm.	300	2200	3.16	25	t, e1, e2, e3, e4, e5
Pleopeltis alansmithii (R.C. Moran) A.R. Sm. & Tejero	2100	3000	33.25	32	(t), dw, r, e1, e2, e3, e4, e5
Pleopeltis angusta Humb. & Bonpl. ex Willd.	600	1800	3.25	8	e1, e2, e3, e4
Pleopeltis astrolepis (Liebm.) E. Fourn.	300	600	1.60	5	e3, e4
Pleopeltis conzattii (Weath.) R.M. Tryon & A.F. Tryon	2500	2500	1.00	1	t
Pleopeltis crassinervata T. Moore	600	2200	7.18	17	e1, e2, e3, e4, e5
Pleopeltis fallax (Schltdl. & Cham.) Mickel & Beitel	500	600	1.67	3	t, dw, e1, e2, e3
Pleopeltis furfuracea (Schltdl. & Cham.) A.R. Sm. & Tejero	500	600	6.25	4	t, e2, e3, e4
Pleopeltis madrensis (J.Sm.) A.R. Sm. & Tejero	2500	2500	4.00	2	e1, e2, e3
Pleopeltis mexicana (Fée) Mickel & Beitel	1000	3000	34.68	34	t, r, e1, e2, e3, e4, e5
Pleopeltis muenchii (Christ) L.D. Gómez	2700	3000	56.15	26	t, dw, r, e1, e2, e3, e4, e5
Pleopeltis plebeia (Schltdl. & Cham.) A.R. Sm. & Tejero	1000	3000	18.06	32	t, r, e1, e2, e3, e4, e5
Pleopeltis polypodioides (L.) E.G. Andrews & Windham	300	1500	8.00	14	t, e1, e3, e4
Pleopeltis pyrrholepis (Fée) A.R. Sm. & Tejero	300	1000	5.10	10	e1, e2, e3, e4, e5

TABLE 1. Continued.

Taxa	Min	Max.	N	F	Life form
Polypodium echinolepis Fée	1300	1500	5.18	11	e1, e2, e3, e4, e5
Polypodium martensii Mett.	2500	2900	34.86	21	(t), e1, e2, e3, e4, e5
Polypodium plesiosorum Kunze	600	600	4.50	2	e2, e4, e5
Polypodium pleurosorum Kunze ex Mett.	1800	2200	9.88	24	t, e1, e2, e3, e4, e5
Polypodium puberulum Schltdl. & Cham.	2000	2200	5.69	16	e1, e2, e3, e4, e5
Polypodium rhodopleuron Kunze	2100	2100	1.50	2	t, e1, e2, e3, e4, e5
Polypodium subpetiolatum Hook.	2700	2700	4.00	2	e1
Polypodium sp.	1000	1000	3.00	1	e1
Serpocaulon falcaria (Kunze) A.R. Sm.	1500	2700	23.39	28	t, r, e1, e2, e3, e4
Serpocaulon triseriale (Sw.) A.R. Sm.	300	1000	3.63	8	t, dw, e1, e2, e3, e4, e5
Stenogrammitis delitescens (Maxon) Labiak	2200	2200	8.00	1	e2
Stenogrammitis hellwigii (Mickel & Beitel) Labiak	2800	3000	21.13	15	r, e1, e2
Stenogrammitis prionodes (Mickel & Beitel) Labiak	1500	2200	9.33	18	e1, e2, e3, e4, e5
**Terpsichore aspleniifolia* (L.) A. R. Sm.	2400	2400	1	1	r
Pteridaceae					
Adiantum poiretii Wikstr.	2500	2500	7.00	2	t, e1
Adiantum pulverulentum L.	300	600	7.35	17	t
Adiantum tenerum Sw.	500	500	2.00	1	t
Adiantum trapeziforme L.	500	500	8.00	1	t
Ananthacorus angustifolius (Sw.) Underw. & Maxon	500	500	3.50	2	e3
Antrophyum ensiforme Hk.in Benth.	1000	2100	6.36	11	e1, e2, e3, e4
**Gaga chaerophylla* (M. Martens & Galeotti) F. W. Li & Windham	1800	1800	1.00	1	t
Jamesonia hirta (Kunth) Christenh	2000	2000	1.00	1	t
Polytaenium chlorosporum (Mickel & Beitel) E.H.Crane	500	1300	2.40	5	e1
Polytaenium feei (W.Schaffn. ex Fée) Maxon	500	600	11.40	5	(t), dw, r, e1, e2, e3
Pteris altissima Poir.	300	600	3.00	4	t, dw
Vittaria bradeorum Rosenst.	1500	2200	2.82	11	dw, e1, e2
Vittaria flavicosta Mickel & Beitel	500	1000	2.89	9	(t) e1, e2, e3, e4
Vittaria graminifolia Kaulf.	2100	3000	30.87	39	(t) dw, r, e1, e2, e3, e4, e5
Saccolomataceae					
Saccoloma inaequale (Kunze) Mett.	1000	2100	12.71	21	t
Tectariaceae					
Tectaria heracleifolia (Willd.) Underw.	300	600	50.63	19	t, r
Thelypteridaceae					
Goniopteris biolleyi (Christ) Pic. Serm.	600	600	1.00	1	t
Goniopteris ghiesbreghtii (Linden) J. Sm.	600	600	6.00	2	t
Goniopteris hatchii (A.R.Sm.) Á. Löve & D. Löve	1500	1500	12.50	2	t, dw
Goniopteris imbricata (Liebm.) Á. Löve & D. Löve	500	500	3.67	6	t
Goniopteris liebmannii (Maxon & C.V. Morton) Salino & T.E. Almeida	300	500	7.14	7	t
Goniopteris obliterata C. Presl	300	300	5.67	3	t

highest total species richness per elevational step was 57 species at 1,500–2,100 m and the lowest at 3,000 m with 15 species.

Within the total of 195 species we found 141 growing as terrestrials and 129 as epiphytes, whereas 75 were strictly terrestrial and 77 strictly epiphytic, which means that 39 species were recorded within both life forms, excluding four hemiepiphytic species. Species with double notations (as terrestrials and epiphytes) mainly belonged to the families Dryopteridaceae, Polypodiaceae, and Hymenophyllaceae. Both major life forms followed different trends, with epiphytic richness per plot mostly about double of the terrestrials, and with a pronounced hump-shaped peak, whereas terrestrials were more or less constant up to 2,500 m and decreasing slightly at higher elevations (Fig. 3).

Epiphytic species on average had a wider elevational range and frequency than terrestrials (elevational range of epiphytes: 510.3 ± 580.8 m and terrestrials: 222.8 ± 351.9 m; Wilcoxon test, $P<0.01$, frequency of epiphytes: 9.61 ± 10.06, both: 10.35 ± 10.21 and terrestrials: 5.77 ± 6.85, ANOVA, $F=4.8$, $df=2$, $P < 0.01$).

All other transects from Bolivia, Ecuador, Costa Rica, and Mexico showed a hump-shaped richness pattern and similar maximum richness at mid-elevations (more or less at the same elevation). Only the transects located at the edges of the tropical realm, in Mexico and the southernmost transect, at Masicurí in Bolivia, presented lower richness values compared with Costa Rica, Ecuador, and the other transect in Bolivia (Fig. 4, ANOVA, $F=20.27$, $df=7$, $P < 0.001$). However, the peak of maximum richness did not decrease with increasing latitude towards lower elevations.

Discussion

With this study, we report the most detailed plot-based study on fern species richness along a complete elevational gradient (300–3,000 m) in humid tropical forest in Mexico. In 121 plots we found 195 species mostly belonging to the families Polypodiaceae, Dryopteridaceae, and Hymenophyllaceae, coinciding with other transects in Mexico (Acebey, Krömer, and Kessler, 2017; Carvajal- Hernández *et al.*, 2017; Krömer *et al.*, 2013), Central and South America (Kluge and Kessler, 2005; Mehltreter, 1995; Salazar *et al.*, 2013). The species recorded in this study represent 29% of the total ferns of the state of Oaxaca in a surface of ca. 95,360 km^2 and 18% of Mexico (Mickel and Smith, 2004), this in only 4.8 ha of sampling. This shows the importance of the Sierra de Juárez, as a national refuge for ferns, since in this sampling area occur more species than in larger areas as the Yucatan Peninsula (ca. 125,000 km^2; integrated for three complete states in Mexico) with only 65 species (Fernández Carnevali *et al.*, 2012) or the state of Tabasco (ca. 24,731 km^2) with 169 species (Carvajal-Hernández *et al.*, 2018).

The highest species numbers were recorded at mid-elevations at about 1,500–2,100 m. This hump-shaped pattern of fern richness is commonly found in tropical mountains and has been explained by climatic factors, especially temperature, precipitation, and fog frequency, e.g., from the Andes (Kessler,

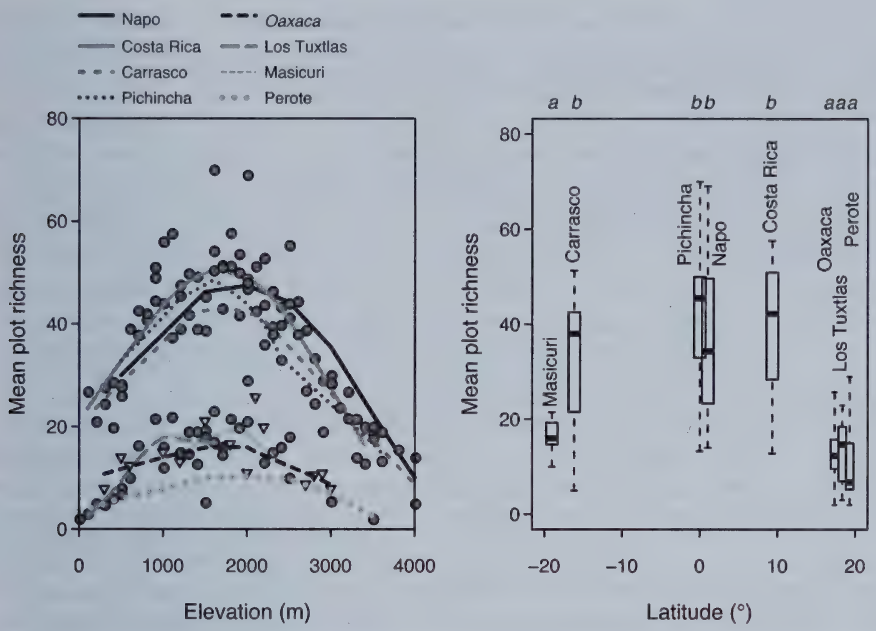

FIG. 4. Left: Patterns of fern species richness along eight elevational transects in the Neotropics, following the latitudinal gradient from Bolivia (-19 and-16° S) via Ecuador (~0°) and Costa Rica (9° N) to Mexico: Los Tuxtlas (17° N) and Cofre de Perote (19° N), Veracruz and Sierra de Juárez, Oaxaca (18° N). Trendlines were set with locally weighted regressions (LOWESS). Right: Mean plot richness plotted against latitude. Letters above indicated significant different groups following analysis of variance (ANOVA) and Tukey post-hoc test ($P < 0.001$).

2001; Krömer *et al.,* 2005; Salazar *et al.,* 2013), Central America (Watkins *et al.,* 2006), Asia (Bhattarai, Vetaas, and Grytnes, 2004; Grytnes and Beaman, 2006), and Africa (Hemp, 2002). However, while species richness counts, especially in the Neotropics, average 40–50 species in plots of the same size as in this study, we found a considerably lower number of fern species at mid-elevations (on average 25 species), which was comparable to nearby transects in Veracruz (Acebey *et al.,* 2017; Carvajal-Hernández *et al.,* 2017; Krömer *et al.,* 2013). Higher species richness is expected in riparian and ravine zones in the Sierra de Juárez, where ferns are more abundant due to humid conditions (Carvajal-Hernández and Krömer, 2015; Kessler, 2010; Kluge and Kessler, 2011). Because this study excluded these habitats, the total number of species is likely to be somewhat higher than found here.

In evaluating life forms, we observed that the elevational hump of species richness was mainly driven by epiphytes. Apart from the extremes at the upper and lower ends of the gradient, this life form had twice the number of species per plot as terrestrial taxa. This has also been reported before (Acebey, Krömer and Kessler, 2017; Kessler, 2001; Kluge and Kessler, 2005; Krömer *et al.,* 2013; Salazar *et al.,* 2013; Watkins *et al.,* 2006) and seems to be a consistent tropics-wide fern richness pattern. In our study, we additionally found that epiphytes had a wider elevational range and were more frequent compared to terrestrial species, as also found for ferns in Bolivia (Kessler, 2002). Dispersal might play a role in the broader distribution of epiphytes, because spores released in the forest canopy are likely to disperse greater distances (Peck, Peck, and Farrar, 1990). Additionally, epiphytic and terrestrial niches operate at largely different scales: whereas terrestrial microhabitats change due to, e.g., soil nutrients, soil moisture, and topographic parameters over distances of several dozen meters (Jones, Szyska, and Kessler, 2011; Cicuzza *et al.*, 2013), epiphytic niche differentiation is driven by small-scale features due to tree crown architecture (Krömer, Kessler, and Gradstein, 2007). Hence, a study plot of 400 m^2 is likely to contain a wider range of epiphytic than terrestrial microhabitats with the consequence that this plot usually contains a higher number of epiphytes with a wider ecological variability.

Probably the most interesting result of our study is that, at least for neotropical ferns, there appears to be no gradual downward shift in species richness with increasing distance from the Equator, but a sudden reduction of species richness at the transitions from the tropics to the subtropics, as already reported by Salazar *et al.* (2013). The causes of this sudden reduction in fern richness are probably related to climatic conditions but may also include a historical and evolutionary signal related to the tropical origin of many major fern groups (Kessler, Karger, and Kluge, 2016).

Although the regional fern diversity of humid montane forests in southeastern Mexico is high, we found that at the local scale it is lower compared with similar ecosystems in Costa Rica, Ecuador, and Bolivia. The confirmation of the Mexican Biogeographic Transition Zone (MTZ) for ferns suggests that this zone is likely a consequence of climatic factors. Colder temperatures and especially the occurrence of frost act as climatically limiting factors of fern distributions with increasing elevation (Bhattarai, Vetaas, and Grytnes, 2004; Kluge, Kessler, and Dunn, 2006) and may be limiting factors in the latitudinal distributions of ferns with increasing distance from the Equator. Moreover, the lack of appropriate data from the latitudinal range between Costa Rica and Mexico indicates that the change in species richness may also either be abrupt further south or even more gradual across Central America. Therefore, more research is needed inside of this transition zone and in Central America to have a complete overview of the latitudinal diversity patterns.

ACKNOWLEDGMENTS

We thank Alan Smith for the priceless support and valuable comments during the process, especially for help in the identification. We thank also the anonymous reviewers and editors for their contributions to improve the manuscript, Municipal authorities, Gary Carlin Ochoa and local guides in Sierra de Juárez to open the access to their communities and forest, Sergio Avendaño and INECOL for the logistic support, Daniel Tejero Díez for contribution in some nomenclatural aspects and Israel Estrada Contreas (INBIOTECA) for their guidance with ArcGIS. This work was supported by a scholarship DAAD-CONACyT (57177537) to the first author in the Länderbezogenes Kooperationsprogramm mit Mexiko.

Literature Cited

Acebey, A.R., T. Krömer, and M. Kessler. 2017. Species richness and vertical distribution of ferns and lycophytes along an elevational gradient in Mexico. Flora 235:83–91. http://dx.doi.org/10.1016/j.flora.2017.08.003.

Alfonso-Corrado, C., F. Naranjo-Luna, R. Clark-Tapia, J. E Campos, O. R. Rojas-Soto, M. D. Luna-Krauletz, and N. Pacheco-Cruz. 2017. Effects of environmental changes on the occurrence of *Oreomunnea mexicana* (Juglandaceae) in a biodiversity hotspot Cloud Forest. Forests 8:261.

Arriaga, L., J.M. Espinoza, C. Aguilar, E. Martínez, L. Gómez, and E. Loa. 2000. Regiones terrestres prioritarias de México. Comisión Nacional para el Conocimiento y uso de la Biodiversidad. México, México.

Bhattarai, K. R., O. R. Vetaas, and J. A. Grytnes. 2004. Fern species richness along a central Himalayan elevational gradient, Nepal. Journal of Biogeography 31:389–400.

Boyle, B. L. 1996. Changes on altitudinal and latitudinal gradients in neotropical montane forests. Cambios en gradientes altitudinales y latitudinales en bosques montanos neotropicales. Dissertation, Ph. D, Washington University, Division of Biology and Biomedical Sciences, St. Louis, Missouri, USA.

Carvajal-Hernández, C. and T. Kromer. 2015. Riqueza y distribución de helechos y licófitos en el gradiente altitudinal del Cofre de Perote, centro de Veracruz, México. Botanical Sciences 93:601–614. DOI: 10.17129/botsci.165.

Carvajal-Hernández, C., T. Krömer, J. C López-Acosta, J. A. Gómez-Díaz, and M. Kessler. 2017. Conservation value of disturbed and secondary forests for ferns and lycophytes along an elevational gradient in Mexico. Applied Vegetation Science 20:662–672. DOI: 10.1111/avsc.12318.

Carvajal-Hernández, C., L. Silva-Mijangos, M. Kessler, and M. Lehnert. 2018. Adiciones a la pteridoflora de Tabasco, México: la importancia del bosque mesófilo de montaña. Acta Botanica Mexicana 124. DOI: 10.21829/abm124.2018.1300.

Cicuzza, D., T. Krömer, A. D. Poulsen, S. Abrahamczyk, T. Delhotal, H. M. Piedra, and M. Kessler. 2013. A transcontinental comparison of the diversity and composition of tropical forest understory herb assemblages. Biodiversity and Conservation 22:755–772. DOI:10.1007/s10531-013-0447-y.

Fernández Carnevali, G.C., J. L. Tapia Muñoz., R. Duno De Stefano, I. M. Ramírez Morillo, L. Can Itzá, S. Hernández Aguilar, and A. Castillo. 2012. La flora de la Península de Yucatán Mexicana: 250 años de conocimiento florístico. CONABIO. Biodiversitas, 101:6–10.

Gentry, A. H. and C. Dodson. 1987. Contribution of non-trees to species richness of a tropical rain forest. Biotropica 19:149–156.

Gradstein, S. R.,M. N. Nadkarni, T. Krömer, I. Holz, and N. Nöske. 2003. A protocol for rapid and representative sampling of vascular and non-vascular epiphyte diversity of tropical rain forests. Selbyana 24:105–111.

Grytnes, J. A. and J. H. Beaman. 2006. Elevational species richness patterns for vascular plants on Mount Kinabalu, Borneo. Journal of Biogeography 33:1838–1849.

Halffter, G. and J. J. Morrone. 2017. An analytical review of Halffter's Mexican transition zone, and its relevance for evolutionary biogeography, ecology and biogeographical regionalization. Zootaxa 4226:1–46.

Hassler, M. 2018. World Ferns: Checklist of ferns and lycophytes of the world (version Feb 2017), in Y. Roskov, L. Abucay, T. Orrell, D.Nicolson, N. Bailly, P. M. Kirk, T. Bourgoin, R. E. DeWalt, W. Decock , A. De Wever, E. van Nieukerken, J. Zarucchi , and L.Penev (eds.), Species 2000 and ITIS Catalogue of Life, 2017 Annual Checklist. Digital resource at www.catalogueoflife.org/annual-checklist/2017. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405–884X.

Hemp, A. 2002. Ecology of the pteridophytes on the southern slopes of Mt. Kilimanjaro–I. Altitudinal distribution. Plant Ecology 159:211–239.

Johansson, D. 1974. Ecology of vascular epiphytes in West African rain forest. Acta Phytogeographica Suecica 59:1–136.

Jones, M.M., B. Szyska, and M. Kessler. 2011. Microhabitat partitioning promotes plant diversity in a mid-elevation tropical montane forest. Global Ecology and Biogeography 20:558–569.

Karger, D. N., J. Kluge, T. Krömer, A. Hemp, M. Lehnert, and M. Kessler. 2011. The effect of area on local and regional elevational patterns of species richness. Journal of Biogeography 38:1177–1185.

Karger, D.N., P. Weigelt, V.B. Amoroso, D. Darnaedi, A. Hidayat, H. Kref, and M. Kessler. 2014. Island biogeography from regional to local scales: evidence for a spatially scaled echo pattern of fern diversity in the Southeast Asian archipelago. Journal of Biogeography 41:250–260.

Karger, D.N., O. Conrad, J.Böhner, H. Kreft, R.W. Soria-Auza, N. Zimmermann, H.P. Linder, and M. Kessler. 2017. Climatologies at high resolution for the earth land surface areas (CHELSA). Scientific Data 4:170122: 1–20 DOI: 10.1038/sdata.2017.122.

Kessler, M. and K. Bach. 1999. Using indicator families for vegetation classification in species-rich Neotropical forests. Phytocoenologia 29:485–502.

Kessler, M. 2000. Elevational gradients in species richness and endemism of selected plant groups in the central Bolivian Andes. Plant ecology 149:181–193.

Kessler, M. 2001. Pteridophyte species richness in Andean forests in Bolivia. Biodiversity and Conservation 10:1473–1495.

Kessler, M. 2002. Range size and its ecological correlates among the pteridophytes of Carrasco National Park, Bolivia. Global Ecology and Biogeography 11:89–102.

Kessler, M. 2010. Biogeography of ferns. Pp. 22–60, in K. Mehltreter, R. L. Walker and J. M. Sharpe (eds.), Fern ecology. Cambridge University Press.

Kessler, M., J. Kluge, A. Hemp, and R. Ohlemüller. 2011. A global comparative analysis of elevational species richness patterns of ferns. Global Ecology and Biogeography 20:868–880.

Kessler, M., D. N. Karger, and J. Kluge. 2016. Elevational diversity patterns as an example for evolutionary and ecological dynamics in ferns and lycophytes. Journal of Systematics and Evolution 54:617–625.

Kluge, J. and M. Kessler. 2005. Inventory of pteridophytes along an elevational transect in Braulio Carrillo National Park, La Selva Biological Station and Cerro de la Muerte, Costa Rica. Brenesia 63:11–33.

Kluge, J., M. Kessler, and R. R, Dunn. 2006. What drives elevational patterns of diversity? A test of geometric constraints, climate and species pool effects for pteridophytes on an elevational gradient in Costa Rica. Global Ecology and Biogeography 15:358–371.

Kluge, J. and M. Kessler. 2011. Influence of niche characteristics and forest type on fern species richness, abundance and plant size along an elevational gradient in Costa Rica. Plant Ecology 212:1109–1121.

Krömer, T., M. Kessler, S. R. Gradstein, and A. Acebey. 2005. Diversity patterns of vascular epiphytes along an elevational gradient in the Andes. Journal of Biogeography 32:1799–1809.

Krömer, T., M. Kessler, and S. R. Gradstein. 2007. Vertical stratification of vascular epiphytes in submontane and montane forest of the Bolivian Andes: the importance of the understory. Plant Ecology 189:261–278.

Krömer, T., A. Acebey, J. Kluge, and M. Kessler. 2013. Effects of altitude and climate in determining elevational plant species richness patterns: a case study from Los Tuxtlas, Mexico. Flora-Morphology, Distribution, Functional Ecology of Plants 208:197–210.

Krömer, T., C. Carvajal-Hernández, A. Acebey, and A. Smith. 2015. A decade of new pteridophyte records for the State of Veracruz, Mexico. American Fern Journal 105:162–175. DOI: 10.1640/0002-8444-105.3.162.

Linares-Palomino, R., V. Cardona, E. I. Hennig, I. Hensen, D. Hoffmann, J. Lendzion, and M. Kessler. 2009. Non-woody life-form contribution to vascular plant species richness in a tropical American forest. Plant Ecology 201:87.

Meave J.A., A. Rincón, and M. A. Romero-Romero. 2006. Oak Forests of the Hyper-Humid Region of La Chinantla, Northern Oaxaca Range, Mexico. Pp. 113-125, in M. Kappelle (eds.) Ecology and Conservation of Neotropical Montane Oak Forests. Volume 185. Springer, Berlin, Heidelberg.

Meave, J. A., A. Rincón-Gutiérrez, G. Ibarra-Manríquez, C. Gallardo-Hernández, and M. A. Romero-Romero. 2017. Checklist of the vascular flora of a portion of the hyper- humid region of La

Chinantla, Northern Oaxaca Range, Mexico. Botanical Sciences 95:722–759. DOI: 10.17129/botsci.1812.

Mehltreter, K. 1995. Species richness and geographical distribution of montane pteridophytes of Costa Rica, Central America. Feddes Repertorium 106:563–584.

Mickel, J. T. and A. Smith. 2004. The Pteridophytes of Mexico Vols. I & II. The New York Botanical Garden Press, USA.

Mickel, J. T. and J. M. Beitel. 1988. Pteridophyte flora of Oaxaca, Mexico. New York Botanical Garden.

Moran, R. C. 1998. The genera of neotropical ferns, a guide for students. Organization for Tropical Studies, San José, Costa Rica.

Parris, B. S., R. S. Beaman, and J. H. Beaman. 1992. The plants of Mount Kinabalu: 1. ferns and fern allies. Kew: Royal Botanic Gardens, Kew. ISBN: 947643354.

Peck, J. H., C. J. Peck, and D. R. Farrar. 1990. Influences of life history attributes on formation of local and distant fern populations. American Fern Journal 80:126–142.

PPG I. 2016. A community-based classification for extant ferns and lycophytes. Journal of Systematics and Evolution 54:563–603. https://doi.org/10.1111/jse.12229.

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rincón-Gutiérrez A. 2007. Estructura y composición florística de los bosque húmedos de montaña de Santa Cruz Tepetotutla, Oaxaca, México. B.Sc. Thesis, Universidad Nacional Autónoma de México.

Rosete-Vergés, F. A., J. L. Pérez-Damián, M. Villalobos-Delgado, E. N. Navarro-Salas, E. Salinas-Chávez, and R. Remond-Noa. 2014. El avance de la deforestación en México 1976–2007. Madera y Bosques 20:21–35.

Rzedowski, J. and R. Palacios-Chávez. 1977. El bosque de *Engelhardtia* (*Oreomunnea*) *mexicana* en la región de la Chinantla (Oaxaca, México). Una reliquia del Cenozoico. Botanical Sciences 36:93–127.

Salazar, L., J. Homeier, M. Kessler, S. Abrahamczyk, M. Lehnert, T. Krömer, and J. Kluge. 2013. Diversity patterns of ferns along elevational gradients in Andean tropical forests. Plant Ecology and Diversity 8:13–24.

Sundue, M. A. 2017. Three new species of grammitid ferns (Polypodiaceae) from the fern hunter's paradise: Sierra Juárez, Oaxaca, Mexico. Systematic Botany 42:160–168.

Watkins Jr. J. E., C. Cardelús, R. K. Colwell, and R. C. Moran. 2006. Species richness and distribution of ferns along an elevational gradient in Costa Rica. American Journal of Botany 93:73–83.

Appendix 1. Location of the study sites along the elevational gradient in Sierra de Juarez, Oaxaca, Mexico.

Locality, Municipality	Elevational band (m)	Coordinates	Type of forest
Cerro Tejón, Paso Nuevo la Hamaca, Valle Nacional	300	17.78685 N, 96.23103 W	Rain Forest
Cerro Marin, Valle Nacional	500	17.82863 N, 96.25282 W	Rain Forest
Cerro Quemado, Vega del Sol, Jacatepec	600	17.79322 N, 96.17304 W	Rain Forest
Yetla, Valle Nacional	1,000	17.72213 N, 96.3089 W	Rain Forest
Puerto Antonio, Comaltepec	1,300	17.67064 N, 96.33496 W	Transition Rain Forest – Cloud Forest
Cerro Redondo, La Esperanza, Comaltepec	1,500	17.62129 N, 96.34547 W	Cloud Forest
Santa Cruz Tepetotutla, San Felipe Usila	1,800	17.71802 N, 96.5587 W	Cloud Forest with *Oreomunnea mexicana*
San Isidro, San Pedro Yolox	2,000	17.60485 N, 96.41625 W	Monodominant *Oreomunnea* Forest
Santa Cruz Tepetotutla, San Felipe Usila	2,100	17.68011 N, 96.55075 W	Cloud Forest
	2,200	17.68041 N, 96.55442 W	Cloud Forest
Jatianguis, Ixtlán	2,500	17.3804 N, 96.51286 W	Pine Forest
Cerro Zacate, San Pedro Yolox	2,700	17.61111 N, 96.53198 W	Oak Forest
Cerro Pelón, Comaltepec	2,800	17.58451 N, 96.51245 W	Oak forest
Cerro Zacate, San Pedro Yolox	2,900	17.61381 N, 96.53339 W	Pine-Oak Forest
Unknown, San Pedro Yolox	3,000	17.60825 N, 96.52305 W	Elfin Forest (tree line)

American Fern Journal 108(3):95–106 (2018)
Published on 16 October 2018

Nutrient and Elemental Composition of Wild Edible Ferns of the Himalaya.

SMRITI CHETTRI
Ph.D Scholar, Department of Horticulture, Sikkim University,
6th Mile, Tadong, Gangtok- 737102, India. Email: chettrismriti20@yahoo.com

S. MANIVANNAN*
Associate Professor, Department of Horticulture, Sikkim University,
6th Mile, Tadong, Gangtok- 737102, India. Email: smanivanan@cus.ac.in

VENKATA RAMANA MUDDARSU
Ph.D Scholar, Department of Horticulture, Sikkim University,
6th Mile, Tadong, Gangtok- 737102, India. Email: venkat.muddarsu@gmail.com

ABSTRACT.—Ferns form a part of vegetable consumption in many communities across the globe. Their popularity is rising because of their high nutritional content such as vitamin C, protein, and iron. Given that the nutritive value of different wild edible ferns has not been explored comprehensively, the present study was carried out. Six different species of wild edible ferns *viz. Diplazium esculentum*, *Dryopteris cochleata*, *Diplazium spectabile*, *Diplazium dilatatum*, *Tectaria coadunata*, and *Diplazium maximum* were analyzed for dietary nutrients and multi-elemental profiling. The highest moisture content (89.83%) and highest vitamin C content (25.38 mg g^{-100}) was found in *Diplazium maximum*.The highest protein was found in *Tectaria coadunata* (12.10%), which was much higher compared to many common commercial vegetables. Fat content was highest in *Dryopteris cochleata* (0.40%), whereas fiber content was found to be maximum in *Diplazium esculentum* (5.05%) and ash content was highest in *Diplazium spectabile* (1.57%). The mineral analysis of these ferns showed that they are good suppliers of sodium (0.46– 3.56 mg g^{-100}), magnesium (6.42– 13.39 mg g^{-100}), and iron (10.36– 16.35 mg g^{-100}). Most of the toxic and heavy metals were found to be within permissible limits. However, some of the fern species collected from certain regions had higher levels of these metals. Based on the present study on dietary nutrients and elemental profile, these edible ferns could be popularized for commercial cultivation. Further, research work could be conducted for standardizing practices and, because of anticipated climate changes, these ferns could add diversity in the world food basket.

KEY WORDS.—elemental profile, green leafy vegetables, heavy metals, ICPMS, wild vegetables.

Among all vegetables, green leafy vegetables have occupied a unique place because of their color, flavor, and health benefits. They are rich sources of β-carotene, ascorbic acid, iron, zinc, folate, and dietary fiber (Negi and Roy, 2000). The nutritional values and mineral content of wild edible plants were richer than that of commercial vegetables and could be used for nutritional purposes (Seal, 2012). Wild plants serve as an indispensable constituent of the human diet, supplying the body with minerals, vitamins, and certain hormone precursors, in addition to protein and energy (Akubugwo, Obasi, and Ginika, 2007). These plants also provide some useful products e.g., medicine, fiber, and fodder (Kayang, 2007). The study of these underutilized vegetables is important not only to identify the potential sources that could serve as alternative food but also to select promising types for domestication. In recent years various lesser known plants are being evaluated for their nutritional and medicinal properties (Bassey *et al.,* 2001; Caldwell, 2010; Pradhan *et al,.* 2015) but there are still large groups of species that are commonly eaten and sold at local market and whose nutritional potential has not been studied. Among wild plants, edible ferns play an important role as they are often included in the daily diet by the population of the Eastern Himalayas.

---

* Corresponding Author

Due to its varied topography, climate, soil, and vegetation, the Himalayan region is very rich in ferns and lycophytes. Out of 362 ferns belonging to 108 genera available in the Sikkim Himalayas (Kholia, 2010) croziers of six species *viz. Dryopteris cochleata* (Buch.-Ham. ex D. Don) C. Chr., *Diplazium spectabile* (Wall. ex Mett.) Ching, *Diplazium maximum* (D. Don) C. Chr., *Diplazium dilatatum* Blume, *Diplazium esculentum* (Retz.) Sw., and *Tectaria coadunata* (J. Sm.) C. Chr. are popularly consumed as vegetables. The versatility of their taste makes them a good selection for vegetable purpose. In the Sikkim Himalayas, croziers of these six species are sold in every local market during each fern's peak season, which varies from March to December. These ferns are considered to possess high amounts of dietary nutrients and other secondary metabolites. However, less effort has been made towards the research and development of these plant species.

Even when research was conducted, it focused primarily on *D. esculentum* (Irawan *et al.,* 2006; Saha, Biswal, and Deka, 2015; Seal, 2012; Tag *et al.,* 2014; Tongco *et al.,* 2014). Therefore, the present research was carried out to discover the nutrient potential of other wild leafy edible ferns.

MATERIALS AND METHODS

Diplazium esculentum, D. cochleata, D. spectabile, D. dilatatum, T. coadunata, and *D. maximum* were the experimental materials in the present study. The research was carried out during the years 2014–2016 in the P.G Laboratory, Department of Horticulture, Sikkim University, Gangtok, India.

Collection of samples.—Fresh young samples (unopened young ferns leaves [croziers] of 20 to 25 cm length) were collected from different localities in the Sikkim Himalayas (Fig. 1) using GPS (Garmin) during the months of June–November 2015 and February–April 2016 (Table 1). The ferns were then stored in fresh sterile polyethelene bags at –20°C until analysis. The identification of the specimens was confirmed by consulting available literature published on ferns and through expert service from the Botanical Survey of India (BSI, India).

Determination of dietary nutrients.—Moisture content of the leaves was estimated by the gravimetric method. Crude fat was determined by essential oil extractor, (Socsplus-SCS 06 DLS, PELICAN, India) by following the solvent extraction method (AOAC, 2000). Crude fiber was analyzed using the fiber estimation system, (Fibra plus-FES 04 AS DLS, PELICAN, India). In addition,

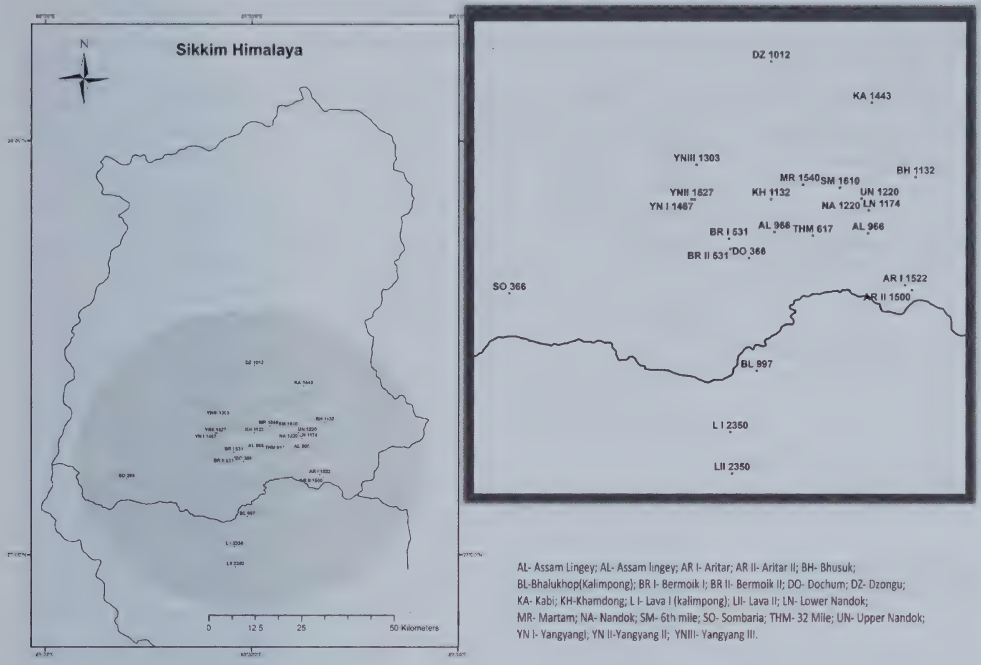
Sikkim Himalaya
N
0
12.5
25
50 Kilometers
DZ 1012
KA 1443
YNIII 1303
BH 1132
MR 1540
SM 1610
YNII 1627
KH 1132
UN 1220
YN I 1487
NA 1220
LN 1174
BR I 531
AL 966
THM 617
AL 966
BR II 531
DO 366
AR I 1522
SO 366
AR II 1500
BL 997
L I 2350
LII 2350
AL- Assam Lingey; AL- Assam lingey; AR I- Aritar; AR II- Aritar II; BH- Bhusuk;
BL-Bhalukhop(Kalimpong); BR I- Bermoik I; BR II- Bermoik II; DO- Dochum; DZ- Dzongu;
KA- Kabi; KH-Khamdong; L I- Lava I (kalimpong); LII- Lava II; LN- Lower Nandok;
MR- Martam; NA- Nandok; SM- 6th mile; SO- Sombaria; THM- 32 Mile; UN- Upper Nandok;
YN I- YangyangI; YN II-Yangyang II; YNIII- Yangyang III.

FIG. 1. Location details of fern collection sites.

ash content was estimated by placing a crucible with dried sample in an electrical muffle furnace (Yorco, India) at 600°C for 6 hours.

Crude protein was determined by Lowry's method (Thimmaiah, 2004) using a UV/VIS Spectrophotometer (Perkin Elmer, Lambda 35 UV/VIS spectrometer). Vitamin C was determined by titremetry (Rangana, 2012).

Analysis of ionomic profiling was carried out by the ICPMS (Perkin-Elmer Nex ion 300X) with cross nebulizer. Digested samples were analyzed for the ionic constitution after standardization of the instrument using the multi elemental standard solutions supplied by the manufacturer for Ag, Al, As, Ba, Be, Bi, Ca, Cd, Ce, Co, Cs, Cu, Fe, Ga, Hg, I, In, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Si, Sr, Ti, U, V, Xe, and Zn analytes.

Statistical analysis.—The treatments were subjected to ANOVA using Completely Randomized Design (CRD) for dietary nutrient analysis using the software STPR. Each species was considered as one treatment and the different collection localities for each species were considered as replications. The number of treatments was six and the replications were four each per treatment.

RESULTS

Ferns were analyzed for proximate constituents *viz.* moisture content, crude fat, fiber, ash, and vitamin C as well as for protein, and the results (Table 2) revealed that edible ferns could act as a supplement for many nutrients in the

Table 1. Location details of Accessions.

Species with harvesting time	Places	Latitude	Longitude	Altitude (m MSL)
Dryopteris cochleata **(March–June)**	Yangyang III	N $27^{0}18.244'$	E$088^{0}24.996'$	1467
	Aritar	N $27^{0}11.176'$	E$088^{0}40.552'$	1522
	Lower Nandok	N $27^{0}17.\ 631'$	E$088^{0}36.\ 443'$	1174
	Assam Lingey I	N $27^{0}15.272'$	E$088^{0}37.30'$	966
Tectaria coadunata **(April – July)**	Nandok	N 27°17. 715′	E$088^{0}36.\ 837'$	1220
	Dochum	N $27^{0}14.508'$	E$088^{0}28.942'$	366
	Sombaria	N $27^{0}10.993'$	E$088^{0}11.864'$	366
	Bermoik	N $27^{0}14.608'$	E$088^{0}28.337$	531
Diplazium dilatatum **(August–November)**	Bhusuk	N $27^{0}35.087'$	E$088^{0}67.658'$	1132
	Dzongu	N $27^{0}27.40'$	E$088^{0}30.42'$	1012
	Lava I	N $27^{0}12085'$	E$088^{0}56.09'$	2350
	Kabi	N $27^{0}24.501'$	E$088^{0}37.561'$	1443
Diplazium esculentum **(March– June)**	Bermoik	N $27^{0}14.608'$	E$088^{0}28.337'$	531
	Khamdong	N $27^{0}17.68'$	E$088^{0}30.42'$	1132
	Yangyang I	N $27^{0}27.68'$	E$088^{0}25.18'$	1303
	Bhalukhop, kpg	N $27^{0}05.522'$	E$088^{0}29.419'$	997
Diplazium spectabile **(August– November)**	Martam	N $27^{0}29.035'$	E$088^{0}55.196'$	1540
	32 Mile, Gangtok	N $27^{0}15.822'$	E$088^{0}33.749'$	617
	Upper Nandok	N 27°17.715′	E$088^{0}36.\ 837'$	1220
	6th mile	N $27^{0}18.495'$	E$088^{0}035.307'$	1610
Diplazium maximum **(October – December)**	Yangyang II	N $27^{0}\ 17.661'$	E$088^{0}24.789'$	1527
	Lava II	N $27^{0}107.741'$	E$088^{0}56.096'$	2350
	Assam lingey II	N $27^{0}15.35'$	E$088^{0}30.653'$	966
	Aritar	N $27^{0}11.245'$	E$088^{0}40.428'$	1500

Table 2. Proximate nutrient content of wild edible fern and its allies.

Species	Moisture (%)	Crude Fat (%)	Fiber (%)	Ash (%)	Vitamin C (mg g^{-100})	Protein (%)
Diplazium dilatatum	87.38 (69.22)	0.18 (2.38)	4.93 (12.81)	1.46 (6.86)	21.25	6.08 (14.05)
Diplazium esculentum	89.34 (70.90)	0.25 (2.74)	5.05 (12.98)	1.33 (6.61)	21.38	3.84 (11.11)
Diplazium maximum	89.83 (71.40)	0.20 (2.52)	3.91 (11.33)	0.98 (5.65)	25.38	5.58 (13.42)
Diplazium spectabile	88.16 (69.83)	0.21 (2.61)	3.39 (10.57)	1.57 (7.10)	21.63	3.56 (10.65)
Dryopteris cochleata	82.23 (66.19)*	0.40 (3.64)	3.77 (11.16)	1.10 (5.99)	23.13	11.84 (20.10)
Tectaria coadunata	85.51 (67.39)	0.18 (2.38)	2.35 (8.74)	1.23 (6.33)	22.25	12.10 (20.27)
GM	87.07 (69.15)	0.24 (2.71)	3.90 (11.26)	1.28 (6.42)	22.50	7.16 (14.93)
CD at 5%	**1.60 (1.57)**	**0.13 (0.75)**	**0.63 (0.98)**	**0.22 (0.60)**	**2.26**	**2.48 (3.14)**

* (The value in parenthesis is in arcsine square root transformed values)
(GM- Grand mean)

TABLE 3. Concentration of dietary minerals in wild edible ferns (mg g^{-100}) (Mean with SD values).

Species	Ca	Cu	Fe	I	Mg	Mn	Mo	Na	Zn
Diplazium dilatatum	1.05± 0.14	19.25± 7.37	10.62± 7.10	0.23± 0.03	13.39± 6.28	6.54± 4.00	3.96± 7.70	1.13± 1.27	0.11± 0.03
Diplazium esculentum	0.66± 0.22	13.37± 5.07	14.38± 16.88	0.19± 0.09	9.56± 3.83	11.91± 14.08	0.19± 0.09	0.50± 0.10	0.10± 0.01
Diplazium maximum	0.98± 0.73	18.76± 17.09	16.35± 17.00	0.17± 0.05	6.42± 4.24	11.05± 5.08	0.99± 1.25	3.56± 6.16	2.84± 5.42
Diplazium spectabile	1.00± 0.23	14.19± 2.14	10.36± 6.57	0.23 ± 0.03	12.00± 2.86	11.12± 5.39	0.48± 0.37	0.46± 0.08	0.23± 0.17
Dryopteris cochleata	0.78± 0.17	12.99± 3.03	15.96± 7.50	0.19± 0.05	12.97± 4.76	20.35± 6.16	0.10± 0.02	0.50± 0.12	0.09± 0.03
Tectaria coadunata	1.09± 0.18	11.29± 2.83	11.01± 3.85	0.23± 0.01	11.87± 3.63	12.00± 3.54	0.16± 0.08	0.55± 0.11	0.12± 0.03

human diet. Other than *D. esculentum*, this is the first report on proximate components and other dietary nutrients of wild edible ferns of eastern Himalayas.

Moisture content.—The results showed that the fronds had high moisture content ranging from 82.23% (*D. cochleata*)–89.83% (*D. maximum*).

Crude fat.—The fat content analyzed ranged from 0.18% (*T. coadunata* and *D. dilatatum*) –0.40% (*Dryopteris cochleata*) and *Dryopteris cochleata* was significantly superior to other species.

Crude fiber content.—*Diplazium esculentum* was found to have superior amounts (5.05%) of crude fiber content followed by *D. dilatatum* (4.93%). These two species had significantly superior fiber content to the rest of the species. *T. coadunata* has the smallest amount (2.35%) of fiber.

Ash content.—Ash content ranged from 0.98% (*D. maximum*) to 1.57% (*D. spectabile*). *Diplazium esculentum* had ash content of 1.33%.

Vitamin C.—The highest amount (25.38 mg g^{-100}) of vitamin C was found in *D. maximum,* whereas *D. cochleata* (23.13 mg g^{-100}) was on par with *D. maximum.* The smallest amount (21.25 mg g^{-100}) was found in *D. dilatatum.*

Protein.—Significantly higher amounts of protein content were found in *T. coadunata* (12.10%) and *D. cochleata* (11.84%) compared to other species. The smallest amount (3.56%) was found in *D. spectabile.*

Dietary minerals.—Multielemental profiling was carried out for all the fern species collected. We analyzed 32 different elements. Only elements that have dietary importance (Table 3) and toxicity to humans (Table 4) are discussed here. National Institute of Nutrition Research, Hyderabad, India has listed 12 elements for human dietary needs (Gopalan, Sastri, and Balasubramanian, 1989) of which three elements were not analyzed and the remaining nine elements are reported here.

The calcium content in the present experiment revealed that it ranged between 0.66 mg g^{-100} in *D. esculentum*–1.09 mg g^{-100} in *T. coadunata*. The copper content was estimated to be lowest in *T. coadunata* (11.29 mg g^{-100}) and highest in *D. dilatatum* (19.25 mg g^{-100}). The present study revealed the

Table 4. Concentration of heavy metals and toxic elements in wild edible ferns (ppm) (mean with SD values).

Species	Al	As	Cd	Hg	Li	Ni	Pb
Diplazium dilatatum	52.9±	18.7±	0.5±	0.01±	1.1±	20.7±	0.2±
	24.4	3.4	0.2	0.01	0.2	13.3	0.1
Diplazium esculentum	58.5±	14.6±	0.4±	0.07±	2.1±	24.5±	0.8±
	29.2	7.6	0.2	0.1	0.9	10.8	0.6
Diplazium maximum	291.7±	16.3±	7.9±	0.4±	25.5±	29.5±	0.3±
	324.1	7.4	14.4	0.7	29.4	20.1	0.1
Diplazium spectabile	39.5±	16.2±	0.6±	0.01±	1.1±	26±	1.3±
	9.9	8.7	0.2	0.02	0.2	2.9	2.1
Dryopteris cochleata	116.9±	18±	0.4±	0.02±	1.6±	57.5±	0.3±
	53.4	0.01	0.1	0.01	0.4	9.1	0.2
Tectaria coadunata	129.3±	15.3±	3.6±	0.01±	1.9±	32.8±	0.5±
	29.5	10	5.4	0.01	0.7	18.2	0.4

iron content in most of the fern species analyzed was usually in the high range, from 10.36 mg g^{-100} *(D. spectabile)* –16.35 mg g^{-100} *(D. maximum).* Three species namely *D. dilatatum, T. coadunata, and D. spectabile* had the highest amount (0.23 mg g^{-100}) of iodine whereas *D. maximum* was estimated to have the smallest amount (0.17 mg g^{-100}). In the present study, magnesium ranged from 6.42 mg g^{-100} (*D. maximum*) –13.39 mg g^{-100} (*D. dilatatum*). The manganese content ranged from 6.54 mg g-^{100} (*D. dilatatum.*) – 20.35 mg g^{-100} (*D. cochleata*). Molybdenum content was estimated to be highest in *D. dilatatum* (3.96 mg g^{-100}) followed by *D. maximum* (0.99 mg g^{-100}) and *D. spectabile.* (0.48 mg g^{-100}) and lowest in *D. cochleata* (0.10 mg g^{-100}) whereas, sodium content was found to be maximum in *D. maximum* (3.56 mg g^{-100}) and minimum in *D. spectabile* (0.46 mg g-^{100}). Among the six edible ferns *D. cochleata* (0.09 mg g^{-100}) had the lowest value for zinc and *D. maximum* (2.84 mg g^{-100}) had the highest value.

Toxic and heavy metals.—The aluminium content in the edible ferns showed relatively higher values. *Diplazium maximum* was estimated to possess maximum Al content (291.7 ppm) followed by *T. coadunata* (129.3 ppm).The arsenic content varied from 14.6 ppm (*D. esculentum*)–18.7 ppm (*D. dilatatum*) in the analyzed species. The cadmium content was highest in *D. maximum* (7.9 ppm) and lowest in *D. esculentum* (0.4 ppm). The mercury content ranged between 0.01 ppm (*D. dilatatum, D. spectabile,* and *T. coadunata*)– 0.07 ppm (*D. esculentum*). Lithium content ranged from *D. maximum* (25.5 ppm)–1.1 ppm (*D. spectabile),* while lead content varied from 0.2 ppm (*D. dilatatum*) –1.3 ppm *(D. spectabile).* Nickel content was in the range of 20.7 ppm (*D. dilatatum)*–57.5 ppm *(D. cochleata).*

Discussion

Moisture content.—Diplazium esculentum had a moisture content of 89.34%, which was similar to the findings of earlier workers with 90.84%

(Irawan *et al.,* 2006) and 91.82% (Tongco *et al.,* 2014). Since there has been no work on other fern species, they were compared with commonly available leafy vegetables (Table 5). The moisture content of other leafy vegetables ranged from 78.66% (Drumstick)–95.43% (Celery). In the present study the moisture content of ferns varied from 82.23% (*D. cochleata*)–89.83% (*Diplazium maximum*), which was comparable with most of the leafy vegetables. The range was nearer to taro leaves (85.66%) and tree fern (88.60%).

Fat content.—Fat content in the present study was in the range of 0.18%–0.40% which were comparable to *Amaranthus* (0.33%), asparagus (0.12%), celery (0.17), coriander (0.52%), lettuce (0.15), ostrich fern (0.40%), and spinach (0.39%). Only taro leaves (0.74%) and drumstick leaves (1.4%) had higher fat content than the analyzed ferns. Earlier reports on the fat content of the *D. esculentum* had reported it as 0.34% (Saha, Biswal, and Deka, 2015) and 0.36% (Pradeepkumar *et al.,* 2015). However, according to Handique (1993) young *D. esculentum* contained very low fat content (0.18%). In the present study, the fat content of *D. esculentum* was estimated at 0.25 %.

Fiber content.—*Diplazium esculentum* had highest fiber content (5.05%). Earlier research on *D. esculentum* by Irawan *et al.,* (2006) and Tongco *et al.,* (2014) reported crude fiber to be 4.82%. In addition Saha, Biswal, and Deka (2015) also reported in the same species the crude fiber to be 4.45%. Among the many leafy vegetables used in the human diet, only *Amaranthus viridis* (6.1%) and curry leaves (6.4%) (Gopalan, Sastri, and Balasubramanian, 1989) had higher fiber content than the *D. esculentum.* All other fern species also had a good amount of fiber, with the lowest being in *T. coadunata* (2.35%). All other leafy vegetables had fiber content from 0.50% (water cress) to 3.70% (taro and tree fern). Hence, all the ferns analyzed had higher fiber content.

Ash content.—Based on the range of ash content (0.98–1.57%) in the ferns that were analyzed, they were as good as *Amaranthus* (1.5%), coriander (1.47%), and water cress (1.20%). In contrast, asparagus, celery, lettuce, and tree ferns had the smallest amount of ash content. However, drumstick, spinach, and taro leaves had higher content among leafy vegetables than the ferns.

Vitamin C.—A comparison with some of the leafy vegetables revealed that the vitamin C content in ferns could be comparable to spinach (28 mg g^{-100}) and coriander leaves (27 mg g^{-100}). Many of the leafy vegetables *viz.* asparagus (5.6 mg g^{-100}), celery (3.1 mg g^{-100}), and lettuce (9.2 mg g^{-100}) had lower vitamin C content than the ferns. The other edible ferns such as Ostrich fern (26 mg g^{-100}) and tree fern (30 mg g^{-100}) had similar values to the ferns surveyed in the present study. Vitamin C content in *D. esculentum* (21.38 mg g^{-100}) was higher than the earlier finding of Irawan *et al.,* (2006). But other reports estimated it to be even higher at 29 mg g^{-100} (Handique 1993) and 32.03 mg g^{-100} (Saha, Biswal, and Deka, 2015).

Protein content.—Ferns in the current study contained high amounts of protein. The highest content was estimated in *Tectaria coadunata* (12.10%) and *Dryopteris cochleata* (11.84%). These values were higher than the protein

TABLE 5. A Comparative Chart of Nutrients in the Common Leafy Vegetables.

Crop	Moisture	Lipid	Fiber	Ash	Protein	Vit.-C	Ca	Cu	Fe	Mg	Mn	Na	Zn
			%						mg g^{-100}				
Amaranth*	91.69	0.33	–	1.50	2.46	43.3	215	0.16	2.32	55	0.89	20.0	0.90
Asparagus*	93.22	0.12	2.10	0.58	2.22	5.6	24	0.19	2.14	14	0.16	2.0	0.54
Celery*	95.43	0.17	1.60	0.75	0.69	3.1	40	0.04	0.20	11	0.10	80.0	0.13
Coriander*	92.21	0.52	2.80	1.47	2.13	27.0	67	0.23	1.77	26	0.43	46.0	0.50
Drumsticks *	78.66	1.40	2.00	2.26	9.40	51.7	185	0.10	4.00	42	1.06	9.0	0.60
Lettuce*	94.98	0.15	1.30	0.62	1.36	9.2	36	0.03	1.20	13	0.20	28.0	0.20
Spinach*	91.40	0.39	2.20	1.72	2.86	28.1	99	0.13	2.71	79	0.90	79.0	0.53
Taro*	85.66	0.74	3.70	1.92	4.98	52.0	107	0.27	2.25	45	0.71	3.0	0.41
*Matteuccia struthiopteris** (Ostrich fern)	–	0.40	–	–	4.55	26.0	32	0.32	1.31	34	–	1	–
Tree fern*	88.60	0.07	3.70	0.06	0.29	30.0	8	0.2	0.16	5	0.54	5.0	0.31
Water Cress*	95.11	0.10	0.50	1.20	2.30	43.0	120	0.08	0.20	21	0.24	41.0	0.11
Dryopteris cochleata	82.23	0.40	3.77	1.10	11.84	23.13	0.78	12.99	15.96	12.97	20.35	0.50	0.09
Tectaria coadunata	85.51	0.18	2.35	1.23	12.10	22.25	1.09	11.29	11.01	11.87	12.00	0.55	0.12
Diplazium dilatatum	87.38	0.18	4.93	1.46	6.08	21.25	1.05	19.25	10.62	13.39	6.54	1.13	0.11
Diplazium esculentum	89.34	0.25	5.05	1.33	3.84	21.38	0.66	13.37	14.38	9.56	11.91	0.50	0.10
Diplazium spectabile	88.16	0.21	3.39	1.57	3.56	21.63	1.00	14.19	10.36	12.00	11.12	0.46	0.23
Diplazium maximum	89.83	0.20	3.91	0.98	5.58	25.38	0.98	18.76	16.35	6.42	11.05	3.56	2.84

* Values were taken from USDA Food Composition Database

content of drumstick leaves (9.40%), taro leaves (4.98%), *Amaranthus* (2.46%) or any of the other leafy vegetables under comparison. Even *D. spectabile,* which had the lowest amount (3.56%) of protein, was better than many leafy vegetables such as amaranth, asparagus, celery, lettuce, and spinach. Comparison with other earlier reported edible ferns revealed that the Himalayan ferns, except *D. spectabile* and *D. esculentum,* generally had a higher amount of protein than the ostrich fern (4.55%) and *Marsilea crenata* (Amoroso, Mendez, and Villalobos, 2017). The protein content in *T. coadunata* and *D. cochleata* was higher than most of the common vegetables such as cabbage (1.8%), cauliflower (5.9%) and even leguminous vegetables such as peas (7.2%) and broad bean leaves (5.6%) (Gopalan, Sastri, and Balasubramanian, 1989). These commercial vegetables are available during the winter, but the edible ferns can be eaten as a protein supplement during the summer. Irawan *et al.,* (2006) estimated protein content in at 2.23% for *D. esculentum.* However, the present value of (3.84%) was lower than the findings of most of the earlier workers *viz.* Seal (2012), Tag *et al.,* (2014), and Tongco *et al.,* (2014).

From the above mentioned observations it could be inferred that among the different species analysed, *D. cochleata* was found to have the highest amount of crude fat (0.40%) and second highest amount of vitamin C. (23.13 mg g^{-100}) and protein (11.84%). In addition, *D. maximum,* which had the highest amount, could be used as a supplement for vitamin C and *T. coadunata* for protein supplement as it had the highest protein content. *Diplazium esculentum* and *D. dilatatum* could be used as a fiber supplement. All the species had a high amount of moisture content. A dietary food having either a high amount of protein or fiber should also have high moisture content. Hence, these edible ferns can offer a diet without any digestive problems.

Dietary minerals.—Analysis of dietary elements revealed that these six analyzed ferns can supplement the daily dietary needs of Cu, Fe, I, Mg, Mn, Mo, and Zn for human beings. However, these ferns had lower amounts of Ca (0.66–1.09 mg g^{-100}) and Na (0.46–3.56 mg g^{-100}).

Calcium content.—In the present study the calcium content (0.66–1.09 mg g^{-100}) was low in all the ferns under study. These values were lower than the calcium content of all the leafy vegetables under comparison (Table 5). Two reasons could be attributed to the low amount of Ca in the analyzed ferns. One, the average annual rainfall of Sikkim Himalaya is 3500 mm. Because of this heavy rainfall, basic elements, especially Ca, is leached and soils in which the ferns are growing are mostly acidic, with low Ca and enhanced Fe and Al (Singh, 2013). Also, high Al in soil interferes with the uptake of Ca (Schmitt *et al.*, 2017).

Copper content.—The Cu content in the analyzed ferns were very high, ranging from (11.29 to 19.25 mg g^{-100}), whereas most of the leafy vegetables ranged from (0.03 to 0.27 mg g^{-100}). The unusually high amount was because ferns tend to accumulate more Al, As, Cd, Cu, Pb, and Zn (Francis and Peterson, 1983; Nishizono *et al.*, 1987; Roccotiello *et al*, 2015; Van *et al.*, 2006)

Iodine content.—Natural vegetable sources of iodine are rare. However, six ferns under study were good suppliers of iodine. The amount of iodine varied from 0.17 to 0.23 mg g^{-100}.

Iron content.—The iron content of the ferns showed relatively higher values than most of the leafy vegetables such as amaranth (2.32 mg g^{-100}), asparagus (2.14 mg g^{-100}), and drumstick (4.00 mg g^{-100}). Even other edible ferns such as ostrich fern and tree fern had lower amounts of Fe. *Diplazium maximum* (16.35 mg g^{-100}) was estimated to have maximum iron content followed by *Dryopteris cochleata* (15.96 mg g^{-100}) and *Tectaria coadunata* (11.01 mg g^{-100}). The high amount of Fe may be because the acidic soils of Sikkim had high amounts of Fe due to leaching of bases. High soil Fe could have contributed to high leaf Fe.

Magnesium content.—Among the different fern species, magnesium content ranged between 6.54–13.39 mg g^{-100}. The values could be comparable with celery (11 mg g^{-100}), asparagus (14 mg g^{-100}), lettuce (13 mg g^{-100}), and taro leaves (5 mg g^{-100}).

Manganese content.—The ferns had high manganese content ranging from 6.54–20.35 mg g^{-100}. Comparatively, the leafy vegetables which ranged from 0.1 mg g^{-100} in celery to 1.06 mg g^{-100} in drumstick leaves, had lower values to these ferns.

Molybdenum content.—Himalayan ferns also showed they are good suppliers of Mo, especially *D. dilatatum.* Compared to common leafy vegetables like amaranth (0.13 mg g^{-100}), coriander leaves (1.12 mg g^{-100}), and spinach (0.01 mg g^{-100}) (Gopalan, Sastri, and Balasubramanian, 1989), these ferns supply very high amounts.

Zinc content.—The zinc content estimated in all the ferns (0.09–0.23 mg g^{-100}) except *D. maximum* were similar to most of the leafy vegetables such as celery (0.13 mg g^{-100}) and lettuce (0.20 mg g^{-100}). Whereas *Diplazium maximum* contained 2.84 mg g^{-100} of Zn. The unusually high amount can be attributed to the accumulation pattern of ferns (Francis and Peterson, 1983; Nishizono *et al.*, 1987; Roccotiello *et al*, 2015; Van *et al.*, 2006).

Sodium content.—Ferns had very low amounts of Na compared to most of the leafy vegetables. Only *D. maximum* (3.56 mg g^{-100}) and tree fern had a little higher range. This was likely due to the soils in which these Himalayan ferns are grown, which are typically acidic and normally had small amounts of Na.

Heavy metals.—The heavy metals accumulated were compared with the CODEX Standard-193 (1995) and it was found that all the ferns accumulated higher amounts of As, Cd, and Al than the permissible limit. With the exception of *D. maximum,* the other fern species accumulated lower amounts of Hg than the permissible limit. In the case of Pb, three species, *viz. D. dilatatum, D. maximum,* and *Dryopteris cochleata* were within the permissible limit. As the Sikkim Himalyan soils were acidic and rich in Al (Singh, 2013), ferns accumulated high Al (Schmitt *et al.*, 2017). In addition, ferns and lycophytes tend to accumulate more amount of Al, As, Cd, Cu, Pb, and Zn (Francis and Peterson, 1983; Nishizono *et al.*, 1987; Roccotiello *et al*, 2015; Van *et al.*, 2006). Hence, more accumulation of these elements in a majority of the species was expected.

Conclusions

These six edible fern analyzed had better nutritional content than some of the commercial vegetables commonly utilized. These edible ferns have great potential to overcome nutritional deficiency especially in hilly areas. The analysis of dietary nutrients showed that the edible ferns were rich sources of protein, vitamin C, and crude fiber. The mineral analysis of these ferns showed that they are good suppliers of Cu, Fe, I, Mg, Mn, Mo, and Zn. In addition, if their accumulation of As, Al, and Cd could be reduced within the permissible limit through cultivation practices, they are safe for consumption. Apart from these advantages, they are inexpensive and one or other species could be made available throughout the year, if their harvesting season could be extended to January and February by cultural practices. Based on the dietary nutrients and mineral content, these edible ferns can be popularized and further research work could be conducted for standardizing practices so as to exploit them at a large commercial scale. In the changing climatic scenario, edible ferns could add to the food basket of human beings.

Literature Cited

Akubugwo, I. E., A. N. Obasi, and S. C. Ginika. 2007. Nutritional potential of the leaves and seeds of black nightshade- *Solanum nigrum* L. var *virginicum* from Afiko-Nigeria. Pakistan Journal of Nutrition 6:323–326.

Amoroso, V.B., R.A. Mendez, and A.P. Villalobos. 2017. Bringing back the lost value of Philippine edible ferns: their antioxidant, proteins and utilization. International Journal of Advanced Research, 5:757–770.

AOAC. 2000. Official Methods of Analysis, 17th edition. Association of Official Analytical Chemists, Washington, DC.

Bassey, M. E., E.U.I. Eluk, M. M. Ibe, and B. A. Ndon, 2001. *Diplaziumsammatii: Athyriaceae ('Nyama Idim')*: Age-related nutritional and antinutritional analysis. Plant Foods for Human Nutrition 56:7–12.

Caldwell, M. J. 2010. Ascorbic acid content of Malaysian leaf vegetables. Ecology of Food and Nutrition 1:313–317.

Codex General Standard For Contaminants And Toxins In Food And Feed. Codex Stan 193-1995. 1–44.

Francis, P. C., and R. L. Petersen. 1983. Effect of copper, cadmium, and zinc on percent spore germination of the cinnamon fern (*Osmunda cinnamomea*) and the sensitive fern (*Onoclea sensibilis*) . Bulletin of Environmental Contamination and Toxicology 30 : 559 – 566.

Gopalan, C., B. V. R. Sastri, and S. C. Balasubramanian, 1989. Nutritive value of Indian foods. National Institute of Nutrition, Indian Council of Medical Research, Hydrabad. Pp. 161.

Handique, A. K. 1993. Free amino acid content in non-conventional leafy vegetables. Crop Research 6:189–193.

Irawan, D. C., C. H. Wijaya, S. H. Limin, Y. Hashidoko, M. Osaki, and I. P. Kulu. 2006. Ethnobotany study and nutrient potency of local traditional vegetables in Central Kalimantan. Tropics 15:441–448.

Kayang, H. 2007. Tribal knowledge on wild edible plants of Meghalaya, Northeast India. Indian Journal of Traditional Knowledge 6:177–181.1

Kholia, B. S. 2010. Ferns and fern-allies of Sikkim. State Biodiversity Board and Botanical Survey of India.

Negi, P. S. and S. K. Roy. 2000. Effect of blanching and drying methods on β-carotene, ascorbic acid and chlorophyll retention of leafy vegetables. LWT-Food Science and Technology 33:295–298.

Nishizono, H., S. Suzuki, and F. Ishii. 1987. Accumulation of heavy metals in the metal-tolerant fern, *Athyrium yokoscense*, growing on various environments. Plant and Soil 102: 65–70.

Pradeepkumar, T., V. Indira, and m. Sanka. 2015. Nutritional evaluation of wild leafy vegetables consumed by tribals in the Wayanad district of Kerala. Proceedings of the National Academy of Sciences, India section B: biological sciences 85:93–99.

Pradhan, S., S. Manivannan, and J. P. Tamang, 2015. Proximate, mineral composition and antioxidant properties of some wild leafy vegetables. Journal of Scientific and Industrial Research 74:155–159.

Rangana, S. 2012. Proximate constituents. Pp. 105–106. Hand book of analysis and quality control for fruit and vegetable products. Tata McGraw- Hill, New Delhi.

Roccotiello, E., H. C. Serrano, M. G. Mariotti, and C. Branquinho. 2015. Nickel phytoremediation potential of the Mediterranean *Alyssoides utriculata* (L.) Medik. *C.* hemosphere 119 : 1372 – 1378.

Saha, J., A. K. Biswal, and S. C. Deka. 2015. Chemical composition of some underutilized green leafy vegetables of Sonitpur district of Assam, India. International Food Research Journal 22:1466–1473.

Schmitt, M., K. Mehltreter, M. Sundue, W. Testo, T. Watanabe, and S Jansen. 2017 The evolution of aluminum accumulation in ferns and lycophytes. American journal of botany 104: 573–583.

Seal, T. 2012. Evaluation of nutritional potential of wild edible plants, traditionally used by the tribal people of Meghalaya State in India. American Journal of Plant Nutrition Fertilization Technology 2:19–26.

Singh, A. K. 2013. Land Resource Management in North Eastern Himalayas., *in* S. Arora, B. S. Negi, S. Bhan, J. S. Bali, V. K. Bharti. Mountain People and Environment Mountain People and Environment. Pp. 66–83. New Delhi, India.

Tag, H., J. Tsering, P. K. Hui, B. J. Gogoi, and V. Veer. 2014. Nutritional potential and traditional uses of high altitude wild edible plants in eastern himalayas, India. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering 8:238–243.

Thimmaiah. 2004. Pp. 58–96. Standard Methods of Biochemical Analysis, Kalyani Publishers, New Delhi.

Tongco, J. V. V., R. A. P. Villaber, R. M. Aguda, and R. A. Razal. 2014. Nutritional and phytochemical screening, and total phenolic and flavonoid content of *Diplazium esculentum* (Retz.) Sw. from Philippines. Journal of Chemical and Pharmaceutical Research 6:238–242.

USDA National Nutrient Database for Standard Reference Release 28.

Van, T. K., Y. Kang, T. Fukui, K. Sakurai, K. Iwasaki, Y. Aikawa, and N. M. Phuong. 2006. Arsenic and heavy metal accumulation by *Athyrium yokoscense* from contaminated soils. Soil Science and Plant Nutrition 52: 701–710.

American Fern Journal 108(3):107–111 (2018)
Published on 16 October 2018

Coryphopteris simulata (Thelypteridaceae), A New Combination For The Massachusetts Fern

Susan Fawcett
Pringle Herbarium, Department of Plant Biology, University of Vermont,
111 Jeffords Hall, 63 Carrigan Drive, Burlington, VT 05405, USA susan.fawcett@gmail.com

Abstract.—Previous hypotheses on the evolutionary affinities of the eastern North American fern species most recently recognized as *Parathelypteris simulata* have included hybrid origin from North American progenitors and disjunctions with east Asian species. Recent phylogenetic evidence indicates that *Parathelypteris sensu* Ching is not monophyletic and that its species belong to two distinct clades, each of which includes members of other widely recognized genera: one of these clades includes the type of *Parathelypteris*, and the second includes southeast Asian and Pacific species of the more recently recognized genus *Coryphopteris* along with some temperate Asian species of *Parathelypteris*. Newly generated *rbcL* data representing the North American taxon *Parathelypteris simulata* places it in the *Coryphopteris* clade, as well. In consideration of ecological and phylogenetic evidence, the new combination *Coryphopteris simulata* is proposed.

Key Words.—Parathelypteris, Thelypteris, *disjunction*, *rhizome morphology*

Relationships among the temperate North American species of Thelypteridaceae and their Asian relatives have puzzled pteridologists for more than a century. The Massachusetts fern, *Parathelypteris simulata* (Davenp.) Holttum, is emblematic of such uncertainty, and disparate ideas about its affinities have been proposed based on morphological and cytological evidence.

In the protolog for *Parathelypteris simulata* (originally described in *Aspidium*), Davenport (1894) considered it morphologically intermediate between *P. noveboracensis* (L.) Ching and *Thelypteris palustris* Schott, and entertained the possibility that it was a hybrid before describing it as a distinct species. Wagner (1963) proposed that *P. simulata* (n=64) indeed was of hybrid origin from *P. noveboracensis* (n=27) × *T. palustris* (n=35), resulting in an allopolyploid with n=62, followed by dysploidization. The hypothesis of an allopolyploid hybrid origin between extant North American progenitors, however, has not been supported by subsequent data. Smith (1971) instead proposed a polyploid origin of *P. simulata* from an ancestor with base number x=32, as then known from the Asian species *Coryphopteris pectiniformis* (C. Chr.) Holttum (Manton, 1955).

Other ideas about relationships among north temperate *Parathelypteris* also have invoked disjunctions between North America and northeastern Asia (see Xiang *et al.*, 2015). These groupings generally ally North American taxa with Asian taxa having gradually reduced proximal pinnae, e.g., *P. nevadensis* (Baker) Holttum and *P. noveboracensis* with *P. nipponica* (Franch. & Sav.) Ching; or ally those that lack gradually reduced proximal pinnae, e.g., *P. simulata* with *P. glanduligera* (Kunze) Ching and *P. japonica* (Baker) Ching (e.g., Ching, 1963; Tryon and Tryon, 1974).

Subsequent phylogenetic analyses (Almeida *et al.,* 2016.; Ebihara, 2011; He and Zhang, 2012, N. Patel, S. Fawcett, M. Sundue, and J. Budke, in prep.) have recovered two distinct clades containing taxa of *Parathelypteris sensu* Ching, with topologies that do not correspond closely with previously proposed disjunctions (Fig. 1). One of these two clades included the type of the genus, *P. glanduligera,* with members of the mostly neotropical genus *Amauropelta* nested within it. The second clade (hereafter, the *Coryphopteris* clade) included all sampled species of *Coryphopteris sensu* Holttum (1971) along with several representatives of *Parathelypteris sensu* Ching, which in turn was sister to the first clade plus members of *Metathelypteris*, an east Asian genus comprising 12 species (PPG I, 2016).

Based on the most recent molecular phylogenetic sampling (N. Patel, S. Fawcett, M. Sundue, and J. Budke, in prep.), *Parathelypteris simulata* is sister to *P. nipponica* within the *Coryphopteris* clade, and may represent a recent disjunction (Fig. 1). This result is based on the inclusion of two widely separated populations of *P. simulata,* which had identical *rbcL* sequences. *Parathelypteris simulata* was resolved as the only North American member of the *Coryphopteris* clade; the other North American *Parathelypteris* species, *P. nevadensis* and *P. noveboracensis*, were recovered in the *Parathelypteris s.s./Amauropelta* clade, in accordance with Almeida *et al.* (2016). This result suggests that lamina shape is not a reliable character for distinguishing between these groups, because *P. nipponica* has gradually reduced pinnae similar to members of the *Parathelypteris* s.s./*Amauropelta* clade, and in contrast with other members of the *Coryphopteris* clade.

Throughout its range in North America, *Parathelypteris simulata* is a plant of low nutrient, acid soils, a habitat preference shared with Old World *Coryphopteris* (Holttum, 1982). A notable difference is that *P. simulata* has a slender creeping rhizome, in contrast to the erect caudices in Holttum's (1976) concept of *Coryphopteris*. Holttum (1976) noted, however, that some of Ching's northern species of *Parathelypteris* — some of which have subsequently been transferred to *Coryphopteris* (Zhang, 2012) — had slender creeping rhizomes. This pattern of creeping rhizomes in temperate taxa and erect caudices in tropical taxa can be observed within other fern genera, for example, in *Diplaziopsis* (including *Homalosorus*) (Price, 1990; Kato and Darnaedi, 1988), and *Diplazium* (eFloras 2018). Furthermore, Holttum (1976) noted that these same northern species of *Parathelypteris sensu* Ching had spore and sporangia morphology like that of *Coryphopteris*, but recognized that "to distinguish them clearly would need more recondite techniques than are available to the herbarium botanist" (p. 20).

Although Holttum made great strides in segregating the tropical members of the *Coryphopteris* clade from *Parathelypteris sensu* Ching, my ongoing phylogenomic research in collaboration with A.R. Smith and E. Sessa, *et al.*, seeks to further assess relationships among these taxa. Additional combina-

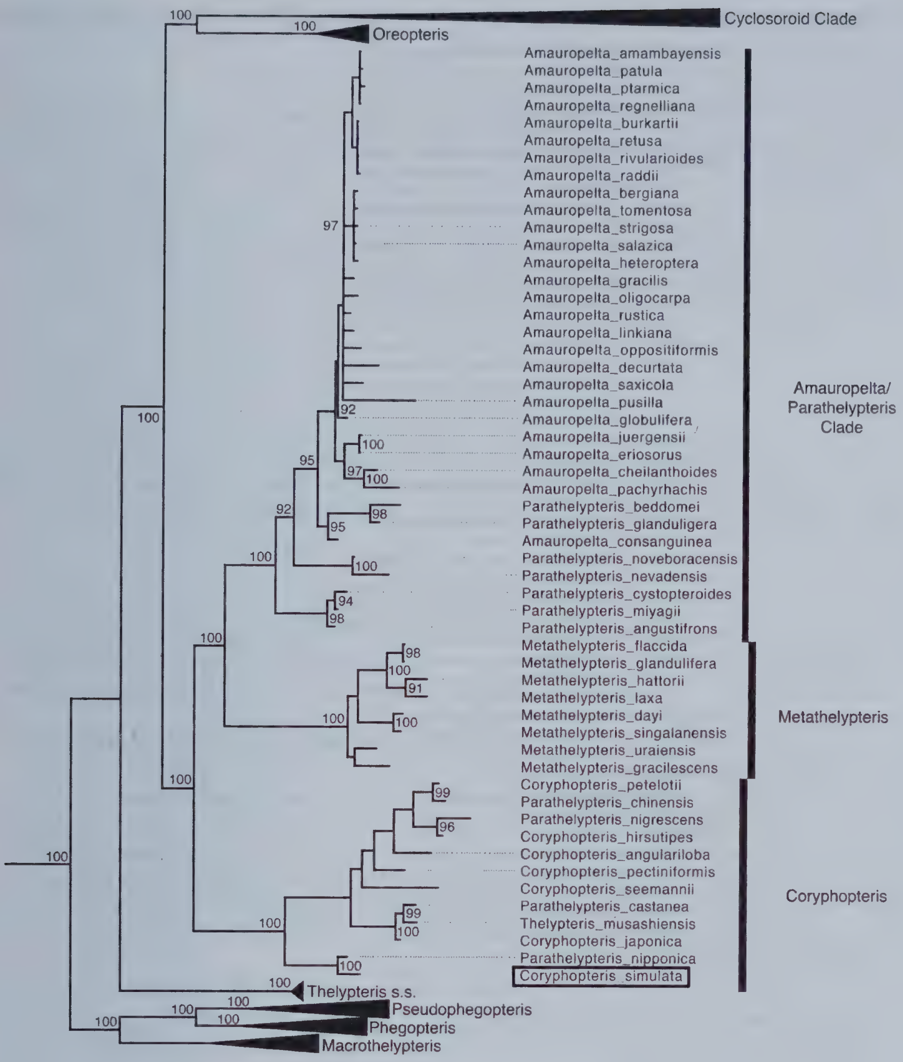

FIG. 1. Bayesian Inference phylogeny based on eleven chloroplast and nuclear loci representing 203 species of Thelypteridaceae (N. Patel, S. Fawcett, M. Sundue, and J. Budke, in prep.). The tree was pruned to demonstrate relationships among members of the *Amauropelta*, *Parathelypteris*, *Metathelypteris* and *Coryphopteris* clades. Posterior probabilities ≥ 90% are shown at corresponding nodes. Note that the position of *Coryphopteris simulata* (in box) within the *Coryphopteris* clade.

tions in *Coryphopteris,* still needed for some Asian taxa, await results of these efforts. Recent evidence firmly places *P. simulata* within the *Coryphopteris* clade, however, and forthcoming publication of regional floras in North America warrants taxonomic updates. In consideration of ecological and phylogenetic evidence, and in the interest of broadening the current circumscription of *Coryphopteris* to include its sole North American member, I propose the following combination:

Coryphopteris simulata (Davenp.) S.E. Fawc., ***comb. nov.*** *Aspidium simulatum* Davenp., Botanical Gazette 19(12): 495. 1894. *Filix simulata* (Davenp.) Farw., Report of the Michigan Academy of Science, Arts and Letters 6: 200. 1904. *Thelypteris simulata* (Davenp.) Niewl., American Midland Naturalist 1: 226. 1910. *Parathelypteris simulata* (Davenp.) Holttum, Webbia 30(1): 195. 1976. *Wagneriopteris simulata* (Davenp.) Á. Löve & D. Löve, Taxon 26: 325. 1977.

Acknowledgments

I thank the members of the Barrington Lab, especially Nikisha Patel, Amanda Hill, and Melita Schmeckpeper for assistance in the lab. Bruce Baldwin, Alan Smith, David Barrington, Michael Sundue, Carl Rothfels and three anonymous reviewers provided helpful comments on the manuscript.

Literature Cited

Almeida, T.E., S. Hennequin, H. Schneider, A.R. Smith, J.A.N. Batista, A.J. Ramalho, K. Proite, and A. Salino. 2016. Towards a phylogenetic generic classification of Thelypteridaceae: Additional sampling suggests alterations of neotropical taxa and further study of paleotropical genera. Molecular Phylogenetics and Evolution 94:688–700.

Ching, R.C. 1963. A reclassification of the family Thelypteridaceae from the mainland of Asia. Acta Phytotaxonomica Sinica 8:289–335.

Davenport, G.E. 1894. Two new ferns from New England. With some observations on hybridity and nomenclature. Botanical Gazette, 19:492–497.

Ebihara, A. 2011. *rbcL* phylogeny of Japanese pteridophyte flora and implications on infrafamilial systematics. Bulletin of the National Museum of Nature and Science, Series B 37:63–74.

eFloras, 2018. *Diplazium* in: Flora of China. Published on the Internet http://www.efloras.org [accessed 12 June 2018] Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA.

He, L.J. and X.C. Zhang. 2012. Exploring generic delimitation within the fern family Thelypteridaceae. Molecular Phylogenetics and Evolution 65:757–764

Holttum, R.E. 1971. Studies in the family Thelypteridaceae. III. A new system of genera in the Old World. Blumea 19:17–52.

Holttum, R.E. 1976. Studies in the family Thelypteridaceae X. The genus *Coryphopteris.* Blumea 23:18–47.

Holttum, R.E. 1982. Flora Malesiana Series II, Pteridophyta. London.

Kato, M. and D. Darnaedi. 1988. Taxonomic and phytogeographic relationships of *Diplazium flavoviride, D. pycnocarpon*, and *Diplaziopsis.* American Fern Journal 78:77–85.

Manton, I., in Holttum, R.E. 1955. A Revised Flora of Malaya. Vol. ii. Ferns of Malaya. Government Printing Office, Singapore.

PPG I. 2016. A community-derived classification of extant lycophytes and ferns. Journal of Systematics and Evolution. 54:563–603.

Price, M.G., 1990. Philippine Fern Notes. Contributions of the University of Michigan Herbarium 17:267–278.

Smith, A.R. 1971. Chromosome numbers of some New World species of *Thelypteris.* Brittonia 23:354–360.

Tryon, A. and R. Tryon. 1974. Geographic patterns in temperate American ferns and some relationships in *Thelypteris.* American Fern Journal 64:99–104.

Wagner Jr., W.H. 1963. A biosystematic survey of United States ferns: Preliminary abstract. American Fern Journal 53:1–16.

Xiang, J.Y., J. Wen, and H. Peng. 2015. Evolution of the eastern Asian–North American biogeographic disjunctions in ferns and lycophytes. Journal of Systematics and Evolution 53:2–32.

Zhang, X.C. 2012. Lycophytes and Ferns of China. Peking University Press, Beijing.

American Fern Journal 108(3):112–116 (2018)
Published on 16 October 2018

SHORTER NOTES

DAVID S. CONANT, 1949–2018.—David Stoughton Conant was born on June 17, 1949 to David Perry Conant and Aune Lepisto Conant. He grew up in Charlestown, New Hampshire, on the banks of the Connecticut River. The Connecticut River runs through David's life as it runs through the New England landscape. As a boy, he spent summers jumping into its swift waters from Charlestown's high bridges. As a young father, he ferried his daughters down its winding reaches on a homebuilt raft. And as a professor at Lyndon State College, he led students on canoe expeditions to explore its many islands.

Dave Conant attended Fall Mountain Regional High School followed by the University of New Hampshire, where he received his Bachelor of Science degree in botany in 1971. At the University of New Hampshire, Dave came under the influence of Albion Hodgdon, a plant systematist with a strong interest in the alpine flora of New England. Hodgdon was Dave's style of mentor, always ready for a high-risk venture after rare plants and at the same time a stickler for high-quality work. It was Hodgdon who encouraged Dave to pursue funding for his first field work in Puerto Rico.

It was also Hodgdon who introduced Dave to his friend Rolla Tryon, a prominent scholar of fern systematics and biogeography at Harvard University. In 1972, Dave began his Ph.D. research at Harvard under the direction of Rolla and his wife Alice, focusing on the ecology and evolution of tree ferns in the Caribbean. On completing his Ph.D. in 1975, Dave sought and won a job in the Upper Connecticut River Valley at Lyndon State College. With him came his partner and soul-mate, Aminta "Minty" Kitfield Conant, whom he met while serving as a laboratory teaching assistant at the University of New Hampshire. Dave spent his academic career at Lyndon State College, and during this time he received multiple National Science Foundation grants. He undertook many high-risk ventures to remote and untraveled places to collect specimens, including Venezuela, Borneo, New Guinea, Australia, the Dominican Republic, Puerto Rico, Costa Rica, Brazil, and Mexico.

At both Harvard and at Lyndon State, Dave's intellectual passion was developing insights into the ecology and evolution of the scaly tree ferns (Cyatheaceae). Dave was a pioneer in applying molecular techniques to uncovering the unique evolutionary histories of these tree ferns, focused on the plants that he encountered in the course of his extensive field work in Puerto Rico. Dave's approach was broad-based: he made significant research contributions in an array of botanical subdisciplines, including reproductive biology, ecology, systematics, and phylogeny. Early in his career, he carried out what is still one of the few documented surveys of the pattern of spore dispersal away from ferns, using radioactive labeling of a Puerto Rican tree fern (Conant, 1978). Having documented hybrids in Antillean Cyatheaceae (Conant, 1975), Dave went on to propose a completely new mode of speciation for the scaly tree ferns, autogamous allohomoploidy, under which new species

Photo from David Ballou, Lyndon State College archives

are produced following hybridization and isolated by self-fertilization (Conant and Cooper-Driver, 1980). At the dawn of the era in which molecular data were brought to bear on the study of plant systematics and evolution, Dave explored the utility of spore storage proteins for interpreting evolutionary divergence in tree ferns (Conant and DeMaggio, 1989). At the same time, he substantiated his allohomoploid hypothesis with gametophyte culture experiments, demonstrating that tree fern hybrids are likely to be selfers (Conant, 1990). The 1990s brought chloroplast-DNA restriction-site analysis to the study of fern evolution; once again Dave was among the first to explore the new technology, applying it to his chosen group, the Cyatheaceae (Stein et. al., 1992; Burke et al., 1993; Conant et al., 1994, 1995; Stein et al., 1997). This work provided the first molecular data supporting Rolla Tryon's interpretation, based on petiole-scale characters, of some of the early events in the evolution of the scaly tree ferns. Major NSF funding made possible Dave's trip to collect scaly tree ferns across several regions of the Old-World tropics, resulting first in a publication on the phylogenetic relationships of the tree ferns of Mt. Kinabalu in Borneo (Conant, 2001). Then, with the advent of DNA sequencing technology, Dave used these new tools to develop a worldwide scaly tree-fern phylogeny, teaming up with a broad group of fern specialists to accomplish his goals (Korall et al. 2006, 2007; Stein et al., 2010). In one of Dave's most recent publications, he brought his work back home to the Connecticut River Valley, contributing a chapter on flora to Rebecca Brown's *Atlas of the Upper Connecticut River Watershed* (Conant, 2009).

As a research mentor, Dave was skillful and generous, welcoming dozens of Lyndon students into his laboratory over a quarter century. Many who had never imagined themselves as researchers experienced their first success under his direction. Some took part in their first scientific meeting traveling with him to the New England Fern Conference at Harvard Forest. Dave was also an inspirational teacher: his enthusiasm for natural science was infectious and his expertise broad. He taught a remarkable array of courses at Lyndon, including *General Biology, Genetics, The Plant Kingdom, Field Botany and Dendrology, Plant Growth and Function,* and *Advanced Systematics.* In later years, he continued to develop new courses, such as *Deep Impact*, a course that focused on the catastrophic events that brought to an end the age of the great reptiles.

Dave's students looked up to him because he was academically demanding and at the same time fair and approachable. Using Vermont's fields and woodlands as his classroom, Dave opened his students' eyes to the diversity of the New England flora. He enticed them with trips to the alpine summit of Mt. Washington, the grassy banks of the Connecticut River, and the dripping cliffs at Job's Pond. He instilled in them a sense of wonder and a love for the Northeast Kingdom of Vermont: he was a master of place-based education before it had a name. Not all Dave's teaching trips were local, however: some were to the fecund wetlands of the Okefenokee Swamp, the shimmering mouth of the Suwannee River, and the forested slopes of Puerto Rican mountains. For a number of Lyndon State College students, Dave's field trips gave them their first look at the world beyond New England. Those trips transformed their perception of the natural world. Many of them went on to become environmental, community, and educational leaders in Vermont and nation-wide. A number of Dave's protégées have followed him into the botanical profession, bringing his passion for ferns to a new generation of students.

Dave was also generous in his service to the profession of botany, serving as President of the New England Botanical Club from 1996–98 and the American Fern Society from 2004–06. Both of those organizations prospered under his leadership.

After retiring from academia, Dave started an excavating business called Homework Construction. Dave had a lifelong enthusiasm for heavy equipment and enjoyed developing this new skillset in later life.

Greater even than his devotion to botany has been Dave's love of the New England landscape, and particularly, of his Vermont hill farm located three miles from the banks of the Connecticut River. Together Dave and Minty stewarded their forests and fields, maintaining a beautiful network of trails and wood roads throughout the property. At work on one of those roads, Dave was killed in a logging accident on June 27, 2018. He is greatly missed.

BIBLIOGRAPHY, DAVID S. CONANT

1. CONANT, D. S. 1975. Hybrids in American Cyatheaceae. Rhodora 77:441–455.
2. TRYON, R. M. and D. S. CONANT. 1975. The ferns of Brazilian Amazonia. Acta Amazonica 5:23–34.

3. Conant, D. S. 1978. A radioisotope technique to measure spore dispersal of the tree fern *Cyathea arborea* Sm. Pollen & Spores 20:583–593.
4. Conant, D. S. and G. Cooper-Driver. 1980. Autogamous allohomoploidy in *Alsophila* and *Nephelea* (Cyatheaceae): A new hypothesis for speciation in homoploid homosporous ferns. American Journal of Botany 67:1269–1288.
5. Conant, D. S. 1983. A revision of the genus *Alsophila* (Cyatheaceae) in the Americas. Journal of the Arnold Arboretum 64:333–382.
6. Conant, D. S. and A. E. DeMaggio. 1989. Immunological comparisons of spore storage proteins: the use of affinity-purified antibodies and western-blotting in probing the relationships of higher taxa of ferns. Biochemical Systematics and Ecology 17:103–107.
7. Conant, D. S. 1990. Observations on the reproductive biology of *Alsophila* species and hybrids. Annals of the Missouri Botanical Garden 77:290–296.
8. Stein, D. B., D. S. Conant, M. E. Ahearn, E. T. Jordan, S. A. Kirch, M. Hasebe, K. Iwatsuki, M. K. Tan and J. A. Thomson. 1992. Structural rearrangements of the chloroplast genome provide an important phylogenetic link in ferns. Proceedings of the National Academy of Sciences 89:1856–1860.
9. Burke, D. H., L. A. Raubeson, M. Alberti, J. E. Hearst, E. T. Jordan, S. A. Kirch, A. E. Valinski, D. S. Conant and D. B. Stein. 1993. The *chlL* (*frxC*) gene: Phylogenetic distribution in vascular plants and DNA sequence from *Polystichum acrostichoides* (Pteridophyta) and *Synechococcus* sp. 7002 (Cyanobacteria). Plant Systematics and Evolution 187:89–102.
10. Conant, D. S., D. B. Stein, A. E. Valinski, P. Sudarsanam and M. E. Ahearn. 1994. Phylogenetic implications of chloroplast DNA variation in the Cyatheaceae. I. Systematic Botany 19:60–72.
11. Conant, D. S., L. A. Raubeson, D. K. Attwood and D. B. Stein. 1995. The relationships of Papuasian Cyatheaceae to New World tree ferns. American Fern Journal 85:328–340.
12. Stein D. B, D. S. Conant and A. E. C. Valinski. 1997. The implications of chloroplast DNA restriction site variation on the classification and phylogeny of the Cyatheaceae. pp.235–254, *In:* Johns R. J. ed. *Holttum Memorial Volume: published to commemorate the centenary of the birth of Professor R. E. Holttum.* Royal Botanic Gardens, Kew, England.
13. Conant, D. S. and D. B. STEIN. 2001. Phylogenetic and geographic relationships of the tree ferns (Cyatheaceae) on Mount Kinabalu. Sabah Parks Nature Journal 4:25–43.
14. Korall, P., D. S. Conant, H. Schneider, K. Ueda, H. Nishida and K. M. Pryer. 2006. On the phylogenetic position of *Cystodium*: it's not a tree fern–it's a polypod! American Fern Journal 96:45–53.
15. Korall, P., K. M. Pryer, J. S. Metzgar, H. Schneider and D. S. Conant. 2006b. Tree ferns: monophyletic groups and their relationships as revealed by four protein-coding plastid loci. Molecular Phylogenetics and Evolution, 39:830–845.
16. Korall, P., D. S. Conant, J. S. Metzgar, H. Schneider and K. M. Pryer. 2007. A molecular phylogeny of scaly tree ferns (Cyatheaceae). American Journal of Botany 94:873–886.

17. Conant, D. S. 2009. Flora. Pp. 98–104, *in* R. A. Brown (ed.), *Where the Great River Rises: An Atlas of the Upper Connecticut River Watershed in Vermont and New Hampshire.* University Press of New England, Hanover, NH.
18. Stein, D. B., C. Hutton, D. S. Conant, C. H. Haufler, and C. R. Werth. 2010. Reconstructing *Dryopteris* "*semicristata*" (Dryopteridaceae): Molecular profiles of tetraploids verify their undiscovered diploid ancestor. American Journal of Botany 97:998–1004.

Species names coined by David S. Conant

Alsophila aminta D.S.Conant, Rhodora 83(833): 149. 1981.

Alsophila auneae D.S.Conant, Journal of the Arnold Arboretum 64: 369. 1983.

Alsophila jimeneziana D.S.Conant, Journal of the Arnold Arboretum 64: 370. 1983.

David S. Barrington and Catherine A. Paris, Pringle Herbarium, Plant Biology Department, University of Vermont, Burlington, VT 05405 USA (dbarring@uvm.edu; cparis@uvm.edu)

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to the study of ferns and lycophytes for publication in the *American Fern Journal*. Manuscripts should be submitted to http://www.editorialmanager.com/afj/. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication. Mandatory fees will be charged for color figures in print and/or in electronic versions of published papers.

Information for authors, including detailed requirements for formatting manuscripts can be found online at: https://www.amerfernsoc.org/american-fern-journal/.

FOR INFORMATION REGARDING AVAILABLE PUBLICATIONS, VISIT THE AMERICAN FERN SOCIETY'S WEBSITE: http://amerfernsoc.org/

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at The American Fern Society, c/o Plant Resources Center, University of Texas at Austin, Main Bldg., Rm 127, 110 Inner Campus Dr. Stop F0404, Austin, TX 78712-1711. Periodicals postage paid at Austin, TX, and additional entry.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. George Yatskievych,Plant Resources Center, University of Texas at Austin, Main Bldg, Rm 127, 110 Inner Campus Dr, Stop F0404, Austin, TX 78712-1711; e-mail: george.yatskievych@austin.utexas.edu

Changes of address, dues, and applications for membership should be sent to the Membership Secretary.

General inquiries concerning ferns should be addressed to the Secretary.

Back volumes are available for most years as printed issues. Please contact the Back Issues Curator for prices and availability. Digital versions of back issues three years or older are available free online through the Biodiversity Heritage Library: http://www.biodiversitylibrary.org/bibliography/43943#/summary

Subscriptions Rates per Volume:

Personal Journal Membership - (includes Journal and Fiddlehead Forum)	$40
Life Membership	$600
Non-professional Membership - (includes Fiddlehead Forum)	$20
Libraries, Herbaria, & Commercial Organizations	$60

Postmaster: Send address changes to American Fern Journal, c/o Plant Resources Center, University of Texas at Austin, Main Bldg., Rm 127, 110 Inner Campus Dr. Stop F0404, Austin, TX 78712-1711.

AMERICAN
FERN JOURNAL

VOLUME 108 NUMBER 3 JULY-AUGUST 2018

Asplenium serratifolium (Aspleniaceae), a New Fern Species from Central Vietnam Based on Morphological and Molecular Evidence
Ke-Wang Xu, Liang Zhang, Ngan Thi Lu, and Li-Bing Zhang 65

Richness Patterns of Ferns Along an Elevational Gradient in the Sierra de Juárez, Oaxaca, Mexico: a Comparison with Central and South America
Adriana Hernández-Rojas, Michael Kessler, Thorsten Krömer, César Carvajal-Hernández, Anna Weigand, and Jürgen Kluge 76

Nutrient and Elemental Composition of Wild Edible Ferns of the Himalaya
Smriti Chettri, S. Manivannan, and Venkata Ramana Muddarsu 95

Coryphopteris simulata (Thelypteridaceae), A New Combination for the Massachusetts Fern *Susan Fawcett* 107

SHORTER NOTES

David S. Conant, 1949-2018 *David S. Barrington and Catherine A. Paris* 112

COVER CAPTION: *Asplenium serratifolium* Li Bing Zhang & K.W. Xu, a new species described from Bac Huong Hoa Nature Reserve, Quang Tri, central Vietnam. Photo credit: Liang Zhang